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Be bold, Mr Varley

MR Eric Varley took over as Britain's Secretary of State for Energy in an almost new government department nine months ago. He had much goodwill behind him as he struggled to absorb the complexities of the nuclear reactor issues and to negotiate the political and industrial minefield of establishing the government's role in the exploitation of North Sea oil. His decisions on these thorny subjects have been tolerably well received. Alas, while he was coping with the issues of energy in the middle distance, his mind seems to have wandered from the ghastly problems of energy for the present. As a result it has taken nine months for anything of substance to emerge from the Department of Energy on the saving of fuel, and more particularly oil. And what has emerged is modest in the extreme:

- Oil price increases to bear more on the motorist: an extra 8p on a gallon is expected soon.
- Speed limits on roads other than motorways to be reduced.
- Buildings (other than homes) to be maintained at not more than 20° C.
- Improvements in thermal insulation standards for new houses.
- Loans to industry for energy-saving investment—up to a national total of £3 million a year.

None of this is likely to strike terror in the hearts of oil producers. Mr Varley eventually hopes to cut energy consumption by 10% but first estimates are that this package will save only 2%. Drivers on Britain's quaint single carriageway roads would be hard pressed to find stretches where they could exceed the new speed limit of 50 miles an hour. So we must assume that there are other more significant ideas in the pipeline.

The regrettable thing is that it has taken so long to get this short distance. Mr Varley has been gathering around him an Advisory Council on Energy Conservation—a surprisingly lengthy process considering the urgency of the problem—and he has had the benefit of the Think Tank's gratuitous advice this last summer. Yet the measures proposed could have been put together on the back of an envelope the day he arrived in his new office. What is more, nine months ago the mood of the nation was such that tough measures, already imposed in some cases last winter, would have been acceptable as long term propositions. Psychologically, Britain was prepared to live with some austerity. But such constraints as there had been were lifted, presumably for supposed political gain, and by midsummer the man-in-the-street's perception of the energy crisis was that a saving was no longer necessary. Not only the man-in-the-street. A high ranking civil servant in the Department of Energy admitted that the only permanent change in his behaviour as a result of last winter's crisis was that he now turned the light off in his railway compartment when he got off the train. It is

doubtful whether the new measures, which are clearly meant to have as much of a psychological as a physical impact, will restore the lost desire to conserve. What is more, there is almost nothing in Mr Varley's package to encourage industry, the big consumer, to economise.

What is needed is some full blooded government intervention. Whether it takes the form of rationing (and surely the Think Tank could with profit be set to develop a fairly sophisticated scheme for discouraging waste) a massive publicity campaign, jawboning of industrial chiefs or substantial price rises, there are ample opportunities for the government to move fairly deeply into the conservation business. To take one example, such propaganda as the government and nationalised industries have produced so far has seemed unbelievably half-hearted. Discreet advertisements in newspapers will persuade nobody. An enormous effort to raise national consciousness is called for, and this will need a large budget and much imagination. As a result of it, the general public might even end up appreciating solutions of the thermal diffusion equation—more than many scientists do at present. And perhaps someone will develop cheap continuously recording thermometers and other cost-monitoring devices.

Ultimately, the government will have to face up to the central message of the energy crisis—that our way of life will have to change if Britain is to remain solvent. The same is true, of course, of all other countries similarly afflicted, but Britain will be the first to suffer if radical measures are not taken soon. It is strange that an external military threat would be perceived immediately and the public called upon to make sacrifices, whereas an economic threat is deemed unsuitable material with which to go to the country and ask for belt-tightening. Mr Varley might be pleasantly surprised by the response if he chanced his arm considerably more.

A hundred years ago



THE process of polishing the lens of the mirror of the great telescope is going on at the French National Observatory by M. Martin. The diameter of the lens is 120 centimetres, and the polisher is a disc of 40 centimetres. The number of men engaged on the polishing is six. They are obliged to stop frequently on account of the great weight of the polisher. An observer placed on the top of the Observatory, at a distance equal to half the focal distance, superintends the polishing process, watching if the image of a light which is placed in a proper position is reflected with sufficient exactness by the mirror below.

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Strategy for survival 2 : the green revolution



David Spurgeon looks at a report soon to be published on what the farmers at the grass roots really feel about the Green Revolution.

ALTHOUGH in simple terms of production gains the Green Revolution has been an undoubted success, it has produced a harvest of reproach as well as praise. Critics have maintained that the introduction of the new high yielding varieties of wheat and rice together with the necessary package of technological practices has helped the rich get richer while the poor get poorer. They predicted that it would lead to the mechanisation of farms, reduction of opportunities for labour, concentration of land holdings and other factors which would increase inequities between rich and poor. All this was said without much in the way of factual support.

Now, at last, some real evidence is beginning to come in from the fields and farms of Asia. It appears in the results—soon to be published—of studies of 2,428 rice farms in 36 villages located in 14 separate areas of six Asian countries.

The research, funded by Canada's International Development Research Centre and carried out through the

International Rice Research Institute and a number of Asian institutions, was to determine just what changes had occurred in the wake of the introduction of the new rice technology. Answers were sought to such questions as: How extensively have the new varieties been accepted? Who is benefiting from the new technology, and in what way? How has the new technology affected employment? What have been the effects on social structure? And what are the major obstacles to further growth in rice production?

One major finding, in case anybody was still in doubt, was that the Asian rice farmer is not a stubborn traditionalist thoroughly resistant to change. Certainly he responded to innovation in a way that would minimise his risk, but respond he did: in 29 of 32 irrigated villages, 90% or more of the farmers reported that they had at least tried the modern varieties (which as used here means varieties introduced since 1965). Adoption rates vary from country to country, but in Pakistan,

for example, the area planted with the new rice varieties was 42% of the total only five years after their introduction into that country.

Another important finding was that both family and hired labour increased (and not the reverse, as predicted) in all of the study sites except in two Pakistan villages, where practically no labour changes were reported. This was so despite the adoption of the tractor in several places. Malaysian villages had the highest proportion of those taking on tractors, but pre-harvest labour decreased there nonetheless. In fact, the villages where labour-saving technology had been most widely adopted after introduction of the new varieties also reported the largest number of farmers who had increased their employment of family and hired labour. Apparently any savings in labour were more than offset by the labour requirements of the technology itself. And the introduction of the new varieties seems to have provided more, rather than less, employment to landless farm labourers.

On the question of increased income from higher yields the evidence varies. Income definitely increased for some farmers: in one area of the Philippines, the value of the farmer's harvest in both wet and dry seasons was almost three times what it was before introduction of the new varieties. Costs of production also increased, however, because more fertilisers and pesticides are required for the new varieties. Even then, farmers' incomes were often greater than they had been.

Yet, subjectively, many farmers did not see themselves as any better off. One researcher pointed out that this was partly because farmers and their families had eaten more rice themselves since the introduction of the new varieties while not seeing this as a form of profit, which of course it is. Another suggested that previous losses had left farmers with long-standing debts that could not be entirely offset by a single season's gains. Several researchers reported specific economic gains as a result of the new varieties and detailed how the gains had been spent.

Consumer goods figured high among purchases made by the successful farmers. These included bicycles and radios, sewing machines, house furnishings and farm implements. Many spent more on food and education than they had before, whereas others paid off debts with their increased profits.

Not all farmers felt the same way about their status after the introduction of the new varieties. Many saw themselves as better off but a few even thought they were in a worse situation.

The views of the researchers also differed. One report, from India, seemed to confirm the critics' opinions.

It maintained that "the new technology helped to tighten the grip of the big farmer on rural economy" and that, although there was no firm evidence of deterioration in living standards of small farmers, small tenants and labourers, "relative distribution of incomes appears to have worsened."

Yet in an analysis of the whole picture, Celia T. Castillo, of the University of the Philippines, said: "From the data, it is obvious that tenants and small farms are better off with modern than with local varieties as far as their own assessment of increase in rice profits and level of living is concerned. Of course, owner-operators and large farmers were much better off than tenants and small farmers. How else could it have been? It is truly asking for a miracle to expect that the new seeds would bring about social equality where centuries have failed to produce a dent on institutional rigidities."

Dr Castillo says emphasis on the relative gains made by the rich and poor farmers has led to a neglect of another issue—farmers' gains relative to where they were before the advent of the new technology. If one becomes preoccupied with the income distribution issue, one is left with the impression that tenants and small farmers would have been better off in terms of incomes and living standards if they had stuck with traditional varieties, she says. And if this were the case there would be no rationale for spreading the new seeds more widely. But it is not the case, according to the data.

In another analysis, Randolph Barker and Teresa Anden, of the International Rice Research Institute, state: "It is clear that a technology that requires more cash inputs will tend to reinforce this [income distribution] inequity in some of the study villages. One cannot, of course, expect technological innovations to correct serious inequities in access to and benefits from resources. The only mitigating step that can be taken is to attempt to reduce cash requirements, particularly for chemicals, by building more resistance and tolerance into the seed itself. Increasing emphasis is being given to this problem in rice research."

The necessity for such research was illustrated in both West Java and the Philippines. In the former, about half the wet season crop was destroyed by gall midge, and in the latter tungo virus caused similar damage. Farmers in Java noticed that attacks were more severe on the available new varieties, so they reverted to local ones, while in the Philippines resistant modern varieties were available—and were used.

Barker and Anden conclude, from the responses of farmers to a question about their preferences, that local

varieties will remain popular in some areas until modern ones are developed that are suitable to local environmental conditions, or until the strong price preference for local varieties changes. They also note that government policy has been a significant factor in determining the speed with which the new varieties have become available and accepted, if such policy has influenced price and availability.

Dr Castillo pointed to another notable finding of the study: "Although high yield capacity is the characteristic most associated with the new varieties, in many villages where adoption has taken place, their yields did not exceed those of the local varieties; they were adopted because of their shorter growing period and non-photoperiodism." Many farmers used the new varieties because they could provide two crops instead of one. This second crop sometimes replaced one of pulses and vegetables, however, and the impact of this on the protein-poor diet of the farm

family will have to be further analysed.

A clear need emerged for improvement of several factors if the full potential of the new varieties is to be achieved: availability of credit and the necessary infrastructure through which seeds, fertiliser and pesticides can be obtained; irrigation and flood control; and suitable government policies regarding price, information distribution, and so on.

The study should provide a good factual base from which to consider not only what changes the new rice technology has made in Asia, but also what changes are needed for the future. Those involved in it consider, however, that an equally important result—if not a more important one—will be the strengthening of social science research in the region. In their eyes it is not just another research project; it is a device for developing a network of relationships among Asian social scientists concerned with similar problems. □



Land preparation and harvest in the Philippines.



international news

ISRAEL seems to have adapted the diplomatic 'leak' to suit the requirements of the Middle East confrontation. Appropriately for a nation which so reveres science and the academic tradition, a vague confirmation of the Israeli nuclear capability was made recently, not through a lobby's 'nark' sloping around the back door of the Knesset, but by the President, himself a scientist, in open discussion with a group of visiting science journalists.

Exactly what President Ephraim Katzir, former chief scientific adviser to the government, meant when he said that Israel could build a nuclear device if necessary "within a reasonable period of time" is open to a variety of interpretations. Nobody would be very surprised to learn that Israel had saved enough plutonium from its French-built Dimona reactor to manufacture about 10 devices of 20 kilotons—that is, big enough to do to Cairo and Amman what was done to Nagasaki. Neither would it be big news to hear that the aviation industry was working on a delivery system and that, meanwhile, the odd bomber has been set aside for the job. (It's unnerving how many times a day you're told that a Volkswagen roof-rack or a fastish camel might serve as a last resort.)

Anyhow, given the speed with which recent Middle East wars have run their course, "within a reasonable period of time" is more likely to mean "we made a batch of components last year" than "we could manage from scratch in a couple of weeks". This much was already taken for granted.

The only real news was that it was Katzir who was laying it on the line and the following day, when journalists tried to telephone the tidings to editors on the continents of Europe and America, the censor tended to cut in on the call and impose a moratorium. The effect of this kind of reaction is, of course, counter-productive, because jaded editors pay rapt attention to stories which other people want to keep quiet, particularly when the other people are military censors. So when the good news was brought (as inevitably it would be from a purpose-built junket for professional scribblers) it received an arguably inflated Press statement. Obliging, an official clarification followed the next day, to the effect that what the President had meant to do was to stress the official line on nuclear arms. Katzir's office

said he had been referring "to the general potential in Israel of scientists and general scientific-technological experience that, objectively, could be implemented if Israel so desired". The statement added that the President had simply "reiterated past pronouncements to the effect that Israel will not be the first to introduce nuclear weapons to the area".



Temple Mount, Jerusalem

Problems in Israel

by John Hall
and Peter Newmark

At this point interpretations diverge. The conspiracy theorists say that having gathered a fair sample of Western journalists who were searching for military implications in every science project they were shown, the titular head of state and founder of the country's major defence research centre is scarcely going to blurt out new information about the biggest state secret he knows. Neither is he the sort of character who needs to have his statements toned down by nervy 'official clarifications'. In other words it was all a fix. Katzir knew very well how his remarks would be read and the official correction was sufficiently ham-fisted to make no difference to the general interpretation that Israel was all set to go nuclear. The intervention of the censor, on this analysis, was all part of the act, just to make sure the story got page space.

The point of the exercise, on this reading, was to spell out plainly to the Arab world that Israel's nuclear-bluff strategy was over at last. The Hebrew University's Middle East conflict scenario studies have shown that the country's intentionally ambiguous nuclear weapons policy has backfired. The idea was that if nobody really knew

whether or not Israel had a bomb everybody would be cautious about attacking. You had one guess on the issue and you couldn't afford a fifty-fifty chance of error. In fact, according to the studies, the effect of the 'ambiguous' policy has been to increase the race by other Middle East countries to acquire nuclear weapons. There have also been two wars along the way. So the set-up was intended to say to the Arabs: "No more mystery. We'll use bombs if we need to", while at the same time avoiding international censure by broadcasting a softer official line.

The second interpretation? President Katzir, who was speaking freely and informally, didn't anticipate that every word he said would be subjected to a semantic analysis which allowed a news-starved reporter to read in an unexceptional remark the story he'd been looking for all week.

- It is traditional for a journalist visiting a foreign land to draw his truisms from the mouths of anonymous taxi drivers, who encapsulate the wisdom of the ages and folkloric charm in a single aphorism which also happens to contain the kernel of the hack's thesis. As if to preserve one's fondest illusions our Rehovot driver settled behind the wheel and volunteered the information that the trouble with scientific research in Israel was that it contained too many chiefs and too few indians. Seventeen per cent of the working population of one million people were academics, and if some of the chiefs left their campuses and became indians in industry, then things would be so much the better, he added, warming to his subject. (Anyone wishing to establish bona fides is referred to Michael Fox, 3a David Shimon Street, Rehovot.)

In confirmation of Mr Fox's assessment, Dr Eliezer Tal, Director of the National Council for Research and Development, also regretted that the country's research effort was top-heavy on basic work carried out in the universities, and getting more so with every year's intake of immigrants. (An average year's 35,000 arrivals include 350 scientists, and an influx of 600 scientists is expected from the Soviet-American deal to release Russian Jews.)

Research and development spending in Israel breaks down as follows: military, 45% of the total; university research (mainly basic) 30%; industrial, 12%; government laboratory work,

10%; others, 3%. Broadly, says Tal, the three main objectives of the government are to secure a better spin-off from the military work which consumes such a vast proportion of the budget, to look for ways in which the excessive amount of university research can be used to support industrial projects, and to encourage industry to buck up and get on with its own research programmes. To this end incentive programmes are under way which weigh the grant distribution system heavily in favour of the researcher who opts for applied work. Tal points to countries like Switzerland and Sweden as examples of top level industrial producers whose excellence reflects their disproportionately high spending on industrial research. It must be remarked, however, that they have thoroughly complacent taxi drivers.

● In a country whose development depends as much on water as on anything else, it is not surprising that the discovery of new sources is followed by ambitious plans. After the war of 1967, and following a combination of biblical clues and intelligence reports, a search for underground water in the occupied Sinai desert led to the discovery of a considerable reservoir. Carbon dating studies show that this water originated 20,000–30,000 years ago and is therefore un replenishable fossil water. But according to Arie Issar, a hydrologist at the Ben-Gurion University of the Negev, sufficient water can be tapped to support the equivalent of 60 new settlements for 50 years. More precisely, he estimates that the output could be 50 million cubic metres a year.

The Desert Research Institute of the Ben-Gurion University, set up in 1973, has a substantial interest in this new water source. Situated at Sde Boker in the Negev Desert, 30 miles south of the main university campus in Beer-

sheva, its scientists are undertaking a wide programme of desert research. They hope to be able to use the water to irrigate large areas of the Negev so that agricultural development can take place.

A combination of basic and applied research has been designed to tackle the problems that this project will have to solve. Apart from the general problems of establishing settlements in the desert and of making life bearable, or preferably attractive, for the settlers, there are more specific questions raised by the quality of the water. It has been found to be both warm (42° C) and somewhat brackish. The institute's scientists claim that the warmth may actually be an advantage when it comes to growing winter crops. The salinity is also unlikely to pose much problem since there are many crops that can cope with the salt levels involved. In addition it is hoped that a programme of plant breeding will yield other and better salt-resistant strains.

Another approach to persuading the desert to support more life than a few Bedouins is that of introducing alien vegetation which will grow even without irrigation. Two promising cases are *jejoba*, an American desert tree whose nuts are a source of high quality wax, and *atriplex*, an Australian fodder crop for grazing animals. Once the Negev is tamed, the planners see no end of exciting possibilities. There are promises, for example, of a series of safari parks teaming with African wild life. But right now it is not altogether clear when the promised water will flow into the area from the reservoirs.

● The decision of UNESCO to cut off aid to Israel was met with both sadness and bitterness in that country. Israel's president, Ephraim Katzir, felt that it was "the beginning of the destruction of civilised organisations" and ex-

pressed sorrow that politically motivated decisions, which were only to be expected in the UN itself had found their way into UNESCO affairs. To which sentiment the Director-General of UNESCO, Amadou Mbaw, effectively replied in a statement that "the delegates to the general conference are government representatives. It is natural that the problems which perturb the world today should find an echo there".

In other words the oil monopoly and the political intricacies of the Middle East are justification for bringing sanctions to bear against the country which has done the most in that area in recent times for education, science and culture. For the alleged claim against Israel, that of endangering and disfiguring religious sites in the course of archaeological excavations in occupied Jerusalem, can not be taken very seriously. It is certainly a difficult job carrying out excavations in Jerusalem where every inch of land seems to be vied for by two or more religions, nowhere more so that at the disputed site outside the Western (Wailing) and Southern Walls of the Temple Mount. There is, however, next to no expert condemnation of the excavations so far undertaken there by the Archaeology Department of the Hebrew University, Jerusalem.

Ironically Israel has actually benefited financially from the UNESCO decision—the UNESCO contribution to the costs of the excavations was only a quarter of the sum paid into UNESCO funds by Israel. And in terms of participation in UNESCO, the position seems to be unchanged, in that Israel's request to join the organisation's European regional group has not been granted. Israelis are disappointed at the lack of reaction from the scientific community abroad but grimly resigned to their increasing isolation. □

Universities in the not-so-red

BRITISH universities have been spared some of the ravages of inflation by the decision of Mr Prentice, Secretary of State for Education and Science, to increase their recurrent grant by £15 million. This brings the total supplementation of the grant for 1974–75 to about £21 million, roughly what the universities had expected for that year on the basis of the rise in costs during 1973, which is how the government does its calculations.

Of course the rate of inflation has gone up since then, so, although the hard-pressed Committee of Vice-Chancellors and Principals (CVCP)

welcomed the announcement, as a help to the universities in their "grave" situation arising from the high continuing rate of inflation, they warn that substantial economies will continue.

The Secretary of State will soon be able to announce the level of grant for the financial years 1975–76 and 1976–77. Universities must know this decision soon say the CVCP as they are already accepting students for 1975.

What will actually happen to the grants for the remaining two years of the quinquennium remains to be seen.

A warning note was struck by Mr Prentice in his announcement when he said that the level of grants in the future would have to take into account the fact that student numbers were not increasing.

Visa for Voronel

Aleksandr Voronel, the Soviet physicist has received an exit visa for Israel.

In spite of official harassment, illicit Sunday seminars of scientists dismissed after applying for visas for Israel continued meeting during the Autumn in Moscow. On November 15, Voronel was told by the KGB that he could have permission to emigrate provided the seminars were discontinued. Voronel replied that he did not see the connection between his leaving the country and the seminars, which would stop only if the whole group were allowed to emigrate. Nevertheless even though the seminars had been continued in the interim, on December 11 Voronel received an exit visa valid until December 24. □

Scandinavian diary

from Wendy Barnaby

PREVENTION may be better than cure, but when it comes to cancers the Swedes are approaching the problem from both angles. January 1, 1975 will see the introduction of two new measures in Sweden, each aimed at reducing the incidence of cancers and related illnesses.

● A revision of maximum allowable concentrations of chemicals in the working environment has recently been published by the National Swedish Board of Occupational Safety and Health. Issued against the background of reports that various workers have contracted illnesses ranging from cancer to giddiness, kidney malfunctions, loss of memory and cramps after working for sustained periods with polyvinyl chloride and a jet fuel (MC77), the new law lists 125 substances and specifies the maximum allowable concentration of each to which workers may be exposed, averaged out over an eight hour day or a period of fifteen minutes. The permissible levels of about 80 of the substances have been lowered, some drastically, and new chemicals have been put on the list. Vinyl chloride used to be allowed in concentrations up to 20 p.p.m. The new level will be 1 p.p.m., the same as that set recently in the United States.

Other substances affected by the new law include arsenic, 20–30 kg of which is used daily in the making of glass and for which concentrations will be lowered from 0.5–0.05 mg per m³, and chromium salts. Used in the tanning of leather and as pigments in anti-rust paints, these will in future be allowed in concentrations of 0.05 mg instead of the present 0.1 mg per m³. The levels of various solvents (acetone, toluene, formaldehyde) have also been reduced, as have octane-raising additives in fuel.

The new law has been criticised at the Health Centre of Stockholm's Institute of Technology, where it has been argued that the new levels are merely repetitions of those now in force in the United Kingdom and the United States and reflect more a balance between economy and hygiene than any facts about the substances cancer-producing properties. In spite of this, the measure will be welcomed by those for whom it will mean safer working conditions.

● Swedes who have contracted brain tumours will after the New Year have the option of treatment by a new fifteen-ton gamma-ray unit recently installed at the Karolinska Hospital. The design of the unit makes it the only one of its type in the world. Gamma

rays from 179 radioactive cobalt (⁶⁰Co) sources encased in a heavily shielded hemispherical central body are focused on to the tumour, which can then be irradiated without significantly damaging the surrounding tissues. Focusing is done with the aid of a collimator helmet arrangement, and is amazingly accurate. Deep-lying tumours and those just under the pituitary body will be particularly susceptible to such treatment. The latter may not themselves be dangerous, but may grow and put pressure on other nerves, affecting the patient's movement and senses. Victims of Parkinson's disease may also find relief. During some operations the patient will be fully conscious, and will be able to communicate with the operator through a built-in two-way radio.

● Norway has recently joined the European Space Research Organisation's Maritime Communications Satellite (MAROTS) programme which began in 1973. Although Norway only has observer status in ESRO, the country's large maritime fleet gives it a manifest interest in a civil maritime communications system. To seal its co-operation Norway will contribute 1.5% of the cost of the project, presently estimated to be of the order of \$80m. Sixty per cent of the total cost is being borne by the UK, with the balance being shared amongst the other participants: Belgium, France, Italy, the Netherlands, Spain, Sweden and West Germany.

The MAROTS system will consist of ground facilities and one satellite in a geostationary orbit, expected to be positioned around 12.5°W. By means of a wide beam the satellite will cover most of the Atlantic Ocean, the western part of the Indian Ocean and the eastern part of the Caribbean region. The satellite, which will have a design life of not less than three years, will be put into orbit by a Delta launch vehicle from Kennedy Space Centre, it is hoped in mid-1977.

When it is operational, MAROTS will provide general communications, improved distress, search and rescue safety (including "all ships" information broadcasts and individual mobile weather routing by satellite) and evaluation of ranging techniques for line of position determination. The information the project will provide will help in the design of a future system, operating globally, intended to improve on MAROTS' functions and to provide satellite capacity to relieve present congestion in the high frequency bands, permit automation of radiotelephone and teleprinter transmissions and cater for services such as high speed data transmission, not presently possible on middle and high frequency levels.

ARC faces money shortages squarely

by Eleanor Lawrence

THE practical effects of the Rothschild Report are now being felt by the research councils generally and by none more so than the Agricultural Research Council (ARC). Introducing the Annual Report for 1973–74, the Secretary of the ARC, Dr W. M. Henderson, was inevitably concerned with the effects of the resulting White Paper on the distribution of government funds for agricultural research, making themselves felt as they do at a time when there is less money all round.

Since 1967–68, as Dr Henderson points out, the annual growth rate of the Science Budget from the Department of Education and Science for all the research councils had fallen from 11.2% to 3.9% and the ARC's share has decreased from 12.2% to 4%. The source of the funds available to the ARC is also changing. In 1973–74, 59% of its budget came from the Science Budget, 23% from the Ministry of Agriculture, Fisheries and Food (MAFF) and 18% from the Department of Agriculture and Fisheries for Scotland (DAFS). The expected proportions in 1974–75 are only 38% from the Science Budget, 46% from MAFF and 16% from DAFS.

The ARC's present programme of work has fitted in with the requirements of MAFF, the 'customer', without too much upheaval and new commissions from the ministry are scheduled to begin next April. Dr Henderson had special praise for the work of the Joint Consultative Organisation, a body made up of representatives of the ministries, universities, the research council and its institutes and units, and the farming community, which was set up to recommend priorities for future work and to evaluate the existing programme.

The tight financial position implies that no new work can be undertaken by the ARC. Although there seems to be no immediate danger of drastic cuts in particular projects, a reduction in the work force seems inevitable. At the moment there is a moratorium on the filling of posts left vacant through retirement or resignation, but Dr Henderson emphasises that this is not a satisfactory answer. "We cannot," he said, "under any circumstances stop the recruitment of a due proportion of good young scientists". He could, however, give no clear indication how this was to be achieved.

One of the most frustrating restrictions will be the inability to inject the necessary money at the 'growing' points where an increase in funding would bring in the best returns. □

correspondence

UNESCO and Trieste

SIR,—Your leader of November 8 was critical of the line you thought the United Kingdom would be taking on the question of the International Centre for Theoretical Physics, Trieste, at the recent 18th General Conference of UNESCO, and attributed this probable attitude largely to Sir Harold Thompson personally.

Now that the General Conference is over, we would like to put on record that Sir Harold Thompson, as the British spokesman in the Science Commission of the General Conference, was representing the views of the Royal Society UNESCO Committee, endorsed by Her Majesty's Government. Britain was not critical of the achievement of the ICTP: indeed the British delegate praised the Centre and its Director. Nor was the United Kingdom delegation seeking the withdrawal of UNESCO funds to the ICTP in the forthcoming biennium; it voted for the budget for the basic sciences, including the full proposed provision for the Trieste Centre in 1975 and 1976.

Along with several other Member States, however, the United Kingdom delegation at the same time supported a resolution, passed unanimously at the 94th Session of the UNESCO Executive Board in May of this year, which stated that:—

"(The Executive Board) . . . Takes the view that Unesco's continued support to the International Centre for Theoretical Physics, Trieste, for the years 1975–76, is acceptable, but is of the opinion that Unesco's relations with and subvention to the Centre should be re-examined carefully in the light of Unesco's total support to basic sciences."

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A. B. COZENS

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Irrationalism and science

SIR,—I agree wholeheartedly with the comments of Martin Raff (*Nature*, December 6). I have been to a number of good conjuring shows in my time and I have invariably found that in a high proportion of the tricks I am not only unable to see how they were done, but I have been unable to understand how they could possibly have been done without an entirely unreasonable amount of joint conspiracy with mem-

bers of the audience. In no case has this led me to believe that novel mental powers were required.

If we were to believe that Mr Geller's achievements went beyond our scientific understanding in the ways that his supporters suggest, there are some very uncomfortable corollaries which have apparently been unnoticed. For example, if he can bend a fork from a distance he could presumably be able to do the smaller mechanical task of putting a kink in an artery.

I am not imputing any undesirable behaviour to Uri Geller, but if other less upright exponents of his art should appear, could they be trusted not to try this on people who were in their way? We are better now at looking for reliable evidence for such things than were the past believers in black magic or present believers in Juju, and could investigate properly the statistics of unexpected coronary thromboses or strokes among the known enemies in the past of suspected people. Having found a number of suspects for which the probability of chance gave $P < 0.001$, we should still have around one in a thousand who would be unjustly indicated as guilty. For further tests the extensive mediaeval literature on witchcraft should be re-examined.

I seem to remember a technique which consisted of throwing the suspect into a pond; if she sank she was innocent and received a Christian burial while if she floated she was dispatched with a silver bullet and buried with a stake through her heart at cross roads.

Since I think the whole suggestion of super-normal powers is nonsense, I am not myself bothered, but those who take the opposite view might well be needing to consider just what legislation would be necessary for dealing with a witch if their case should ever be proved.

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Strong, attractive, vain . . .

SIR,—The recent contribution by Gribbin¹ suggests, *inter alia*, that there may be a significance in the titular characteristics of the Leo sub-group on the *Nature* editorial staff. This possibility of significance is given weight by McIntosh, who writes of those born under Leo: "The Sun rulership gives him a strong attractive personality and makes it easy for him to command loyalty from others. These qualities

make him a natural leader, and the sign has long been associated with kings and potentates"². It should perhaps also be mentioned that, according to the same source, "The faults that sometimes afflict the Leonian are vanity, pomposity and greed for power".

In view of the fit between sub-group attainment and expectations, it is regrettable that further research, carried out in an institution similar to the one in which Gribbin works, has shown that the Pisces hypothesis does not hold water³.

MARTIN SHERWOOD

New Scientist,
London, UK

¹ Gribbin, J., *Nature*, **252**, 534 (1974).

² McIntosh, C., *The Astrologers and their Creed*, 130 (Hutchinson, London, 1969).

³ *New Scientist*, **64**, 880 (1974).

Ghost authors

SIR,—Ghost authors (*Nature*, December 6) are no new phenomenon. A good many years ago a paper by the late Professor Martin Rushton was published, giving after the author's name his qualification M.A.Cantab. Subsequently this was mistaken for a second author, and the reference "Rushton and Cantab" was a standing joke among dental research workers for some years. It found its way into at least one textbook.

DOROTHY A. LUNT
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Nullius in verba

SIR,—R. B. Cater's complaint (*Nature*, November 29) that 70.4% of all inquiries for a reprint spelt his name wrongly, is not as bad as he makes out.

It is a firm principle in science that the worth of any man's work is independent of that man's non-intellectual attributes, such as mere name. Far from displaying a lack of observational powers I would say that they are verifying the principle (and the motto of the Royal Society)—*nullius in verba*—on the words of no man.

Dr Cater would have had more cause for complaint if either (a) his work was disregarded because he was thought to be insignificant or (b) his work was accepted because his father was thought to have been a good scientist.

COLIN PRICE

The University,
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news and views

Neuropharmacology of peptides

SEVERAL amino acids, such as glutamate, aspartate, glycine and gamma-aminobutyrate, have powerful excitatory or inhibitory effects on neuronal firing in the mammalian central nervous system. Recent findings have shown that certain peptides also have highly specific neuropharmacological actions, and it seems possible that some neurones may release peptides which have a neurotransmitter or modulatory role in the nervous system.

It has been known for some time that neurones in certain regions of the hypothalamus contain and release peptides with important endocrine functions. Thus neurones in the hypothalamus synthesise the hormones vasopressin and oxytocin which are released into the blood stream from the terminals of these cells in the posterior pituitary gland. Nicoll and Barker (*Brain Res.*, **35**, 501; 1971) found that vasopressin exerted potent inhibitory effects on the firing of neurones in the supraoptic nucleus, and suggested that vasopressin might be released by recurrent collateral fibres mediating inhibition of the neurosecretory cells. Vasopressin in low concentrations was also found to cause long lasting changes in pacemaker potential activity in a specific neurosecretory neurone of the land snail *Otala lactea* (Barker and Gainer, *Science*, **184**, 1371; 1974). Other hypothalamic neurones secrete 'releasing factor' peptides into the portal circulation to the anterior pituitary and these peptides represent the final link whereby neural influences control the endocrine activity of this gland.

Recent findings suggest, however, that the neuropharmacological activity of certain hypothalamic releasing factor peptides may not be confined to the endocrine functions of the hypothalamic-pituitary system. The tripeptides TRH (thyrotropin releasing hormone) and MIF (melanocyte inhibitory hormone inhibitory factor) have a wide variety of behavioural effects in man and animals. In animals these peptides potentiate the psychomotor stimulant actions of L-dopa (Plotnikoff *et al.*, *Science*, **178**, 417; 1972) and enhance the behavioural excitation produced by a combination of tranlycypromine and L-tryptophan (Green and Grahame-Smith, *Nature*, **251**, 524; 1974), and accelerate the rate of turnover of noradrenaline and dopamine in the brain (Friedman *et al.*, *Science*, **182**, 831; 1973; Keller *et al.*, *Nature*, **248**, 528; 1974). Several groups have claimed that TRH has transient mood elevating effects in depressed patients and in normal subjects (Prange *et al.*, *Lancet*, **i**, 999; 1972; Tiwary *et al.*, *Lancet*, **ii**, 1086; 1972; Wilson *et al.*, *Lancet*, **ii**, 43; 1973; Wilson *et al.*, *Archs. gen. Psychiatr.*, **29**, 15; 1973). The suggestion that TRH may have a role in controlling neuronal excitability in the central nervous system, apart from its endocrine function, is also supported by the recent report that more than two thirds of the total TRH in rat brain is located in brain regions outside the hypothalamus, with particularly high concentrations in certain septal and pre-optic areas (Winokur and Utiger, *Science*, **185**, 265; 1974; Brownstein *et al.*, *Science*, **185**, 267; 1974).

Another peptide with powerful neuropharmacological effects is the undecapeptide substance P, discovered by von Euler and Gaddum in 1931 in extracts of brain and small intestine (*J. Physiol., Lond.*, **72**, 74; 1931). The amino acid

sequence of substance P purified from bovine hypothalamus was reported by Chang *et al.* (*Nature*, **232**, 85; 1971) and the synthetic material is now available. In this issue of *Nature* (page 734) Konishi and Otsuka show that substance P has a potent excitatory action on mammalian spinal cord motoneurones. They used an isolated spinal cord preparation from newborn rats, in which substances can be applied to the perfusion fluid in precisely controlled concentrations, and found that substance P was about 200 times more potent than the powerful excitant amino acid L-glutamate in causing a depolarisation of motoneurones. Substance P also facilitated monosynaptic spinal cord reflexes. In previous studies Konishi and Otsuka had shown (*Brain Res.*, **65**, 397; 1974) that substance P and the related peptides physalaemin and eledoisin were potent excitants of motoneurones in frog spinal cord, the latter compounds being 1,500–2,000 times more potent than L-glutamate. Takahashi *et al.* (*Brain Research*, **73**, 59; 1974) also confirmed earlier reports that substance P is present normally in bovine dorsal root nerves, and that its concentration in these sensory nerves was 10–30 times higher than in the ventral root spinal motor nerve. These findings thus strongly support the view that substance P may be selectively concentrated in sensory nerves and that it could function as the transmitter substance released from sensory nerve terminals in the spinal cord, as suggested earlier by Lembeck (for review see Lembeck and Zetler, *Int. Rev. Neurobiol.*, **4**, 159; 1962). Substance P is also present in various regions of the brain, and in brain homogenates the peptide is highly localised in nerve terminal particles, or synaptosomes (Whittaker, *Progr. Biophys. molec. Biol.*, **15**, 39; 1965). When applied iontophoretically from microelectrodes on to single neurones, in the cerebral cortex or cuneate nucleus, substance P has been found to be a powerful excitant of neuronal firing (Phillis and Limacher, *Expl. Neurol.*, **43**, 414; 1974; Krnjevic and Morris, *Can. J. Physiol. Pharmacol.*, **52**, 736; 1974). The latter authors, however, found the actions of substance P to be slow in onset and long-lasting, and suggest that the substance is unlikely to represent the quick acting transmitter released from sensory nerve terminals.

The possibility that 'peptidergic' neurones may exist, releasing peptide neurotransmitter substances, or that peptides may exert other long-term modulatory influences on neuronal function is one to delight the neuropharmacologist and the pharmaceutical industry. With modern techniques of peptide chemistry it should be possible to design and test a large number of structural analogues of the naturally occurring materials, and perhaps to develop peptides with highly selective neuropharmacological actions. For example, it may be possible to dissociate the behavioural and endocrine effects of substances such as TRH and MIF, or to obtain analogues of substance P that will penetrate more readily into the central nervous system from the circulation. If the initial promise is sustained, this seems likely to become an important new area for neuropharmacological research.

L. L. IVERSEN

Competition for laser fusion?

from a Correspondent

THE incident laser power needed to achieve a significant thermonuclear energy gain in a deuterium-tritium (DT) target which initially has a density near that of the solid state has been variously estimated to lie between 10^{13} and 3×10^{15} W; the lower power is appropriate to hollow spherical pellets seeded with materials having a nuclear charge greater than unity, and the higher power to pure, homogeneous spherical DT pellets. The predicted power needed to maximise thermonuclear gain from a given target depends not only on the sophistication and ingenuity of numerical or analytical models, but also on various physical assumptions; these may range from the technological, such as the inclusion of two-dimensional effects induced by small variations in incident light intensity or target symmetry, to the fundamental, such as a self-consistent treatment of non-linear laser-plasma phenomena. (For a good introduction to the subject, the reader is referred to review papers by J. Nuckolls, R. E. Kidder, K. A. Brueckner, R. L. Morse, and others, to be published in *Laser Interaction and Related Plasma Phenomena*, 3, edit. by H. Schwartz, Plenum Press.)

An interesting example of the interrelation between practical problems and more fundamental effects in laser fusion is afforded by recent radio-frequency and numerical experiments reported by Kim *et al.* (*Phys. Rev. Lett.*, 33, 886; 1974), in which the trapping of radio-frequency electric fields within a density cavity, or 'caviton', has been demonstrated. Their experiment simulates the generation of a parametric instability which occurs when a non-uniform laser-plasma is driven by a (pump) electric field directed parallel to the density gradient. Although this field component would be zero in an ideally symmetrical

spherical implosion, it may not be negligible in a practical experiment; the applied field is thus enhanced by linear, resonant conversion to electrostatic modes in regions near the critical density.

A wide range of other laser-plasma effects are also of interest. Consequently predictions of the laser energy needed for significant thermonuclear gain depend on the wavelength (λ) of the laser light. For example, Kidder has estimated that, if core-corona decoupling is the limiting physical process in pure DT pellets, laser energy requirements range from 70 kJ (at $\lambda = 0.265 \mu\text{m}$) to 3 MJ (at $\lambda = 10.6 \mu\text{m}$), whilst Nuckolls computes that a 100 kJ, 10^{13} watt, $\lambda = \frac{1}{2} \mu\text{m}$ laser is required for useful power generation (invited paper given at VIII International Quantum Electronics Conference, San Francisco, June 1974). An economic DT fusion reactor thus requires lasers having energies significantly above those possible at present and with efficiencies of some 15% (Spalding, *Energia Nucleare*, 21, 176; 1974), although hybrid fusion-fission energy schemes may lower the required laser efficiency (Horoshko, *et al.*, *Annals of Nuclear Science and Engineering*, in the press). Probably for these reasons, Professor E. Teller recently told an audience at Stanford University that critical experiments in laser fusion may take a generation to complete (*New Scientist*, 64, 502; 1974).

Competition to lasers as a heat source for target compression in inertial confinement experiments comes from charged-particle beams, which can now be generated with very high electrical efficiencies. Considerable energies have already been delivered to small targets. Typical parameters for some present-day laser and relativistic electron-beam target-compression experiments are compared in Table 1. Assuming that ablation of a given target is self regulating, with energy deposition governed by the penetrating power of hot coronal electrons, the absorbed power requirement should be

comparable for both laser and particle-beam approaches to inertial confinement. A fundamental problem for the particle approach may thus prove to be the generation of sufficiently high power beams, and their subsequent application at high, uniform intensities to sub-millimetre targets. Winterberg has recently made the interesting suggestion that 1–10 MV heavy ions, having an energy distribution which is instantaneously mono-energetic but which varies with time, might provide a convenient means for axial (time) compression of the beam after passage through a neutralising drift tube (*Nature*, 251, 44; 1974). Although 300 keV proton beams of approximately 75 J energy have recently been produced with an efficiency of 40% (*Bull. Am. phys. Soc.*, Series II, 19, 871; 1974; papers 2C 14 and 15) no experimental attempt at pulse compression of heavy ion beams has yet been reported. If suitable high-intensity ion-beam techniques could be developed and successfully demonstrated in a compression experiment, the relatively high efficiency with which power could be deposited in the target might provide strong competition to other inertial-confinement approaches.

In summary represent laser techniques seem to offer the most immediate prospect for generating very high density plasmas. Significant laser developments will however be required to permit high-efficiency heating of isolated fuel targets, and to face a potential challenge from charged particle beam techniques for practical fusion energy applications. The likely time scale and scientific challenge to both approaches closely parallels current controlled thermonuclear reactor magnetic confinement programmes.

Probing nuclear band structure

from P. E. Hodgson

MANY nuclei have low-lying states that are readily interpreted as members of a rotational band. Their energies follow quite closely the values expected from the quantum-mechanical theory of a rigid rotator, and their spins follow typical rotational sequences like 0^+ , 2^+ , 4^+ ... or $\frac{1}{2}^+$, $3/2^+$, $5/2^+$... depending on the spin of the lowest member of the band. Furthermore, the transition probabilities for the excitation and de-excitation of these bands are found to be in good accord with the same quantum-mechanical theory.

Many such bands have now been found, especially in the regions of the periodic table far from the closed shells where nuclei are frequently deformed. But though it may be easy to identify the lower members of a band it is

Table 1 Some typical target compression experiments

Beam characteristics	Number of beams	Total energy (J)	Incident power (W)	Incident intensity (W cm^{-2})	Ref. §
1* $\lambda = 1.06 \mu\text{m}$	2	230	2.0×10^{11}	4.0×10^{15}	F1
2* $\lambda = 1.06 \mu\text{m}$		1,000–3,000			F3.2
3* $\lambda = 1.06 \mu\text{m}$	4	800	2.0×10^{12}	2.0×10^{16}	F4.2
4 $\lambda = 0.53 \mu\text{m}$	4	140	—	—	F4.2
5* $\lambda = 1.06 \mu\text{m}$	12	1,400	4.0×10^{11}	—	F4.3
6* $\lambda = 1.06 \mu\text{m}$	4	320	1.5×10^{11}	10^{15}	F4.4
7* $\lambda = 1.06 \mu\text{m}$	2	50	1.7×10^{12}	—	F4.5
8† $\lambda = 10.6 \mu\text{m}$	1	200	1.4×10^{11}	7.0×10^{14}	F4.5
9‡ $\frac{1}{2}$ MV electrons	1	1,000	$\sim 5.0 \times 10^{10}$	$\sim 10^{12}$	F2.1
10‡ 1 MV electrons	2	20,000	$< 10^{12}$	—	F2.2

* Nd-glass laser efficiency $< \frac{1}{4}\%$

† CO_2 laser efficiency currently of order 2% (can be improved)

‡ Electron beam efficiency of order 50%

§ Paper number from the Fifth Conference on Plasma Physics and Controlled Nuclear Fusion Research, IAEA-CN-33, 11–15 November 1974, Tokyo

often difficult to identify the higher members as there may be several states of the appropriate spin with approximately the right excitation energy. The identification is frequently made more difficult by quantum-mechanical mixing of the states of the same spin belonging to different bands that perturbs the simple sequence of energies.

Some recent work by Anantaraman and colleagues at the University of Rochester (*Phys. Rev. Lett.* **33**, 846; 1974) has shown that the $(^6\text{Li}, d)$ reaction can sometimes be used to assist the identification of members of a particular band. Previous work had already shown that this reaction is sensitive to the transferred orbital angular momentum, indicating its usefulness in nuclear spectroscopy. This means that when a nucleus is bombarded with ^6Li ions many deuterons are emitted and the angular distributions of the deuterons of a particular energy leaving the residual nucleus in a particular state is characteristic of the orbital angular

momentum L given in the reaction to the target nucleus. If the target has zero spin, the orbital angular momentum couples vectorially to the spin of the transferred particle giving the spin of the final state $J=L\pm\frac{1}{2}$. This ambiguity can usually be resolved by shell model considerations, or by other measurements, such as that of the polarisation of the emitted particle. If the spin of the target nucleus is not zero several orbital angular momenta can contribute and they can easily be separated if they have similar magnitudes and different shapes, as is often the case. As before this determines or sets limits to the spin of the final state.

The Rochester group studied the reaction $^{25}\text{Mg}(^6\text{Li}, d)^{28}\text{Si}$ to states of ^{28}Si . It was already known that the rotational model is able to describe some of the low-lying states of ^{28}Si by assuming that they belong to oblate $K=\frac{1}{2}^+$ and $3/2^+$ rotational bands. The $K=\frac{1}{2}^+$ band is based on the ground state and has members $\frac{1}{2}^+$ (0.0 MeV), $3/2^+$ (2.43

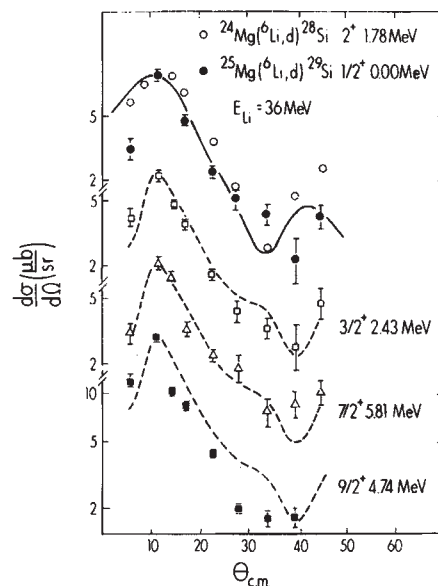


Fig. 1 Differential cross sections for the $^{24}\text{Mg}(^6\text{Li}, d)^{28}\text{Si}$ reaction to the 2^+ state at 1.78 MeV and for the $^{25}\text{Mg}(^6\text{Li}, d)^{28}\text{Si}$ reaction to the states of ^{28}Si forming the $K=\frac{1}{2}^+$ band. The full curve is a distorted wave calculation of the cross section of the first reaction, and the dashed line is a smooth curve drawn through the data for the second reaction to the $\frac{1}{2}^+$ state. All these reactions thus have the angular distribution characteristic of $L=2$ transfer.

Glow discharge optical spectroscopy

from John Walker

TRANSISTORS, integrated circuits, solid state lasers and many other electronic devices are made by introducing impurities into silicon, germanium and compound semiconductors like gallium arsenide. The impurities are usually diffused in at a high temperature or are ion-implanted (fired into the material at a high speed after being accelerated by an electric field). In either case the impurity atoms are not distributed uniformly throughout the semiconductor crystal—their concentration varies with depth. The precise variation (the 'profile') needs to be known, because it affects the production yield and the quality of the device. In general, profiles cannot be calculated accurately: they must be measured. Although various techniques are available, in one technologically important case, boron in silicon, they are either not applicable, too inaccurate, or complicated and expensive.

To resolve this problem a group of workers at the University of Illinois (Greene *et al.*, *Applied Physics Letters*, **25**, 435; 1974) have devised a modification of the well-known technique of spectrographic analysis which they call 'glow discharge optical spectroscopy' (GDOS). The boron-doped silicon sample is bombarded by argon ions in an electrical discharge. This knocks atoms out of the sample, which are then excited in the discharge and emit light of particular wavelengths. Just as sodium street

lamps give out an orange light characteristic of sodium atoms, so a boron discharge emits ultraviolet light at a particular wavelength. The more boron atoms there are in the silicon crystal the more intense the light emitted. Furthermore the crystal surface is gradually worn away by the boron ion bombardment, so that the change in boron concentration with depth can also be investigated.

When the method was applied to a diffused sample the measured boron profile was found to depart from the theoretical distribution by a factor of ten at some depths, confirming the inadequacy of calculations. A second silicon wafer, into which boron had been ion-implanted, had a maximum concentration at a depth of 430 nm, in agreement with calculations, but some boron had penetrated much further into the crystal than the simple theory predicted—an observation also made by other workers.

These results indicate the effectiveness of GDOS in detecting and profiling boron impurity in silicon. The method can be used on other impurities, and on compounds. For example, it has been applied to tin in gallium arsenide. Insulators can also be tested, and impurities need not be electrically active. The equipment is relatively simple, and available in most laboratories. Glow discharge optical spectroscopy obviously has a bright future, particularly in the electronics industry.

MeV), $5/2^+$ (2.03 MeV) and $9/2^+$ (4.74 MeV), and the $K=3/2$ band has members $3/2^+$ (1.27 MeV), $5/2^+$ (3.07 MeV), $7/2^+$ (4.08 MeV), $9/2^+$ (5.65 MeV) and $11/2^+$ (7.14 MeV). The energies are not in regular sequences due to the considerable band mixing. There is a missing $7/2^+$ member of the $K=\frac{1}{2}^+$ band and there are several possible candidates, but it was not known which is the member of the band and which belong to quite different configurations.

Studies of the angular distributions of the deuterons from the reactions that go to each state in the $K=\frac{1}{2}^+$ band showed that they are all the same and have the characteristic shape of an $L=2$ transfer. This shape is known from studies of the $^{24}\text{Mg}(^6\text{Li}, d)^{28}\text{Si}$ reaction to the 2^+ state at 1.78 MeV, which can only go by $L=2$ transfer. The $^{25}\text{Mg}(^6\text{Li}, d)^{28}\text{Si}$ reaction to the $\frac{1}{2}^+$ ground state of ^{28}Si can also go only by $L=2$ transfer, because the spin of ^{25}Mg is $5/2^+$, but all the other reactions to higher members of the $K=\frac{1}{2}^+$ ground state band can in principle go by several different values of L . Thus for example the reaction to the $3/2^+$ state can go by $L=2$ or 4, as these are possible solutions of the vector equation $5/2 + L = 3/2$ (the odd values of L are excluded by the requirement of parity conservation). Nevertheless as shown in Fig. 1 only the lowest $L=2$ transfer is found in this and in all the other transitions

to this band. Among the possible candidates for the $7/2^+$ member of the band is the state at 5.81 MeV that shows just the same angular distribution; this is suggestive but not conclusive as not all the $7/2^+$ states were resolved in the experiment, so other candidates are possible.

In sharp contrast to this uniform $L=2$ behaviour the angular distributions of the $^{25}\text{Mg}(^6\text{Li}, d)$ reaction to states of the $K=3/2^+$ band, shown in Fig. 2, have the irregular behaviour characteristic of a mixture of the allowed L -values. The curves in the figure correspond to various mixtures of L -values, as determined by the $^{25}\text{Mg}(^6\text{Li}, d)$ reaction. This sharp difference in the behaviour of the reactions to these two bands is all the more remarkable in view of the strong mixing between the two bands.

The mixing of the L -values for the transitions to the $K=3/2^+$ band shows that the predominance of $L=2$ in the transitions to the $K=1/2^+$ band is not due to kinematics but must therefore be due to differences in nuclear structure. At present there is no explanation for this behaviour, and it shows the need for more detailed nuclear structure studies.

It is, however, not necessary to understand the reason for the different behaviour of the transitions to the two bands in order to use it empirically as a way of identifying the different bands. It is likely that many other bands will be found to have characteristic signatures, and that these reactions will provide a useful way of studying the structure of rotational nuclei. It will also stimulate nuclear reaction calculations to try to understand the difference in the structures of the bands that give rise to this effect.

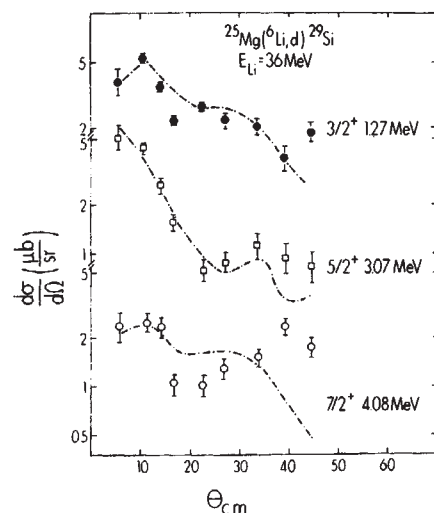


Fig. 2 Differential cross sections for the $^{25}\text{Mg}(^6\text{Li}, d)$ reaction to the states of ^{29}Si forming the $K=3/2^+$ band. The curves for the $3/2^+$ and $7/2^+$ states are mixtures of $L=2$ and $L=4$ distributions and that for the $5/2^+$ state is a mixture of $L=0$ and $L=2$ distributions.

Structure of molecules in solution

from J. Feeney

To reach an understanding of biological recognition processes such as those involved in enzyme-substrate or hormone-receptor interactions will require a detailed knowledge of the three-dimensional structures and the potential flexibility of the component molecules. Inspired by this challenge, Dr R. E. Richards, FRS and Professor R. J. P. Williams, FRS organised a meeting on November 21 at The Royal Society to discuss the present status of the various experimental methods for determining structures and conformations in solution. Although it is clear that none of the available methods offer the high precision of structural information provided by X-ray diffraction, some of these methods allow one to answer the important question of whether the unique conformation present in the crystal is the only one present in solution. Several speakers explored the applications of nuclear magnetic resonance (NMR) spectroscopy to these problems and this method emerged from the discussion as the dominant technique for providing conformational, dynamic and ionisation information for molecules in solution.

R. J. P. Williams (University of Oxford) illustrated how the conformation of ligands such as nucleic acids and peptides can be obtained from the chemical shift and relaxation time perturbations caused by the binding of a paramagnetic lanthanide ion to the molecule. When the interactions can be shown to be dipolar in origin then the chemical shift perturbations depend on r^{-3} where r is the internuclear distance between the lanthanide ion and the perturbed magnetic nucleus (proton, carbon, phosphorus) and an orientational term $(3 \cos^2\Theta - 1)$ where Θ is the angle between this internuclear vector and the principal symmetry axis of the complex. Relaxation changes when using a lanthanide ion such as Gd^{3+} are isotropic and depend on r^{-6} .

A computer-simulated NMR spectrum is then generated for the fixed conformation which best fits the observed data. Only if there is an excellent fit for both the chemical shift and relaxation time perturbations can one assume that there is predominantly a single conformation in solution. It became clear from the discussion that it is possible by careful experimentation to demonstrate the validity of the approach for rigid molecules. But for potentially flexible molecules such as peptides, the 'conformation' as formed by this technique is not simply related to the distribution of conformations

but probably reflects the most populated conformation.

J. Feeney (National Institute for Medical Research, London) described an alternative NMR method of obtaining conformational information involving the well-established procedure of relating measured three-bond H-H spin-coupling constants ($^3J_{\text{HH}}$) to the dihedral angle ϕ about the central bond. When the form of the $^3J_{\text{HH}}/\phi$ relationship (the so called Karplus equation) is known for a particular system it can be used to define dihedral angles in molecules of fixed conformation. For flexible molecules it is possible to estimate the fractional populations from the averaged coupling constants and this allows one to check potential energy calculations. The method has been extended to three bond coupling constants involving other nuclei (^1H - ^{13}C , ^1H - ^{31}P and ^1H - ^{15}N). Various applications to cholinergic neurotransmitters and hormonal peptides were considered. It is clear that for small molecules which are potentially flexible a combination of the spin-coupling constant approach with the lanthanide induced shift measurements is required to obtain the maximum amount of conformation information.

For larger molecules such as the enzyme lysozyme where one expects a unique conformation in solution, the full potential of the lanthanide induced shift technique can be realised. C. M. Dobson and I. D. Campbell (University of Oxford) described how the spectral perturbations caused by binding lanthanide ions to hen egg white lysozyme in solution could be interpreted in terms of the crystal structure of lysozyme, thus indicating that the solution and solid state conformation are very similar. By the elegant use of NMR difference spectroscopy most of the proton resonances in close proximity to the lanthanide ion binding site can be assigned with confidence. Dr Campbell showed how many other proton assignments in lysozyme can be made independently of the X-ray diffraction crystal structure. By using high magnetic fields and computational methods of resolution enhancement many of the proton resonances could be resolved and assigned from a consideration of their multiplet structures, and results from double resonance methods, pH titrations, chemical modifications, paramagnetic probes and inhibitor binding. On the basis of these experiments it is clear that this method can be extended to proteins where the X-ray structure is not yet available. As pointed out by Campbell and Dobson, the assigned resonances may be used to monitor interactions between catalytically important groups, to determine

their ionisation states and to deduce the mobilities of the side chains of certain residues.

T. G. Spiro (Princeton University) showed how resonance Raman spectroscopy of haem proteins can be used to detect deviations from planarity of the porphyrin ring and other structural consequences of changing the spin and oxidation states in the haem groups. The vibrational modes containing this information exhibit greatly enhanced Raman scattering when an electronic transition to which they are coupled is irradiated with laser excitation.

X-ray and neutron scattering techniques were considered by J. E. Enderby (University of Leicester) and J. W. White (University of Oxford). Because the neutron diffraction scattering length for the proton is opposite in sign to that for deuterium, by using mixtures of H_2O/D_2O it is possible to achieve high contrast between the scattering from the solvent and from the solute molecules. For inelastic scatter-

ing there is a large difference in cross section between protons and deuterons which allows observation of the dynamic behaviour of individual components in the various phases of amphiphile/water systems. Low angle neutron diffraction studies on hydrated collagen and tobacco mosaic virus solutions lead to information about the radii of gyration of the molecules in solution.

From measurements of the quasi-elastic spectrum of laser light scattered from macromolecules in solution, G. B. Benedek (Massachusetts Institute of Technology) showed how accurate diffusion coefficients can be obtained. When considered together with sedimentation data these values provide molecular weights. The size and shape of the macromolecules can also be deduced. Interesting differences in the apparent volumes of lysozyme when denatured by different methods (guanidine and heat) were observed but no evidence for intermediate states in the unfolding process could be detected.

The technique is capable of monitoring aggregation of macromolecules and this was well illustrated by a study of the manner in which the aggregation of glutamate dehydrogenase controls the amount of enzyme in its active form.

Most of the techniques considered have still to reach their full potential in their application to structure determination in solution and rapid progress can be anticipated over the next few years.

Huntington's chorea and GABA

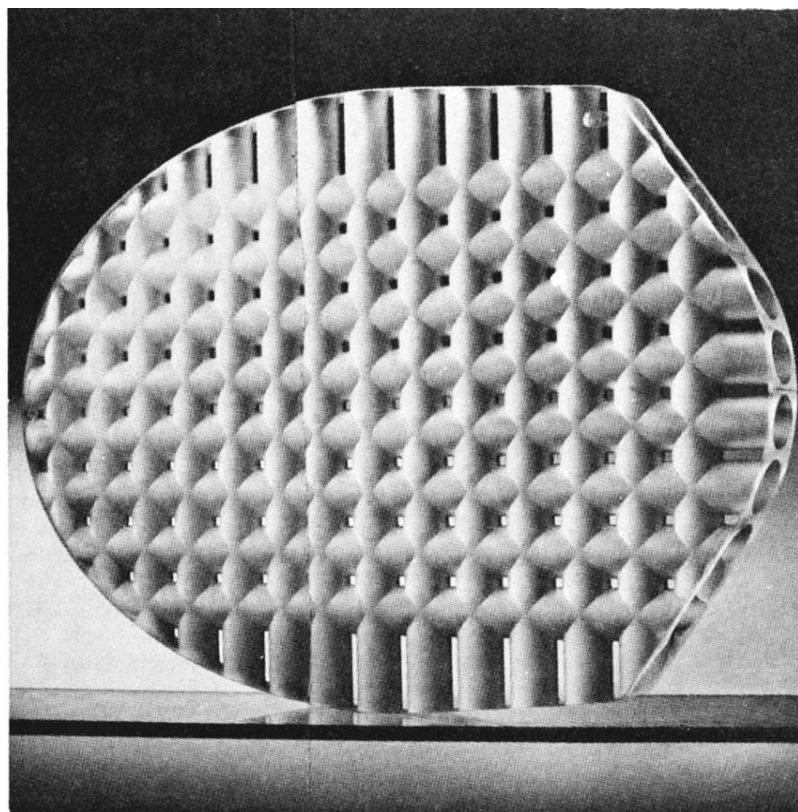
from T. J. Crow

THE apparent diversity of chemical neurotransmitter substances (there are now at least seven with good credentials) in the brain has encouraged the search for specific neurohumoural disturbances underlying neurological diseases of obscure aetiology. Ehringer and Hornykiewicz' discovery (*Klin. Wschr.*, **38**, 1236-1239; 1960) of a deficiency of dopamine in the basal ganglia in Parkinson's disease led to the introduction of L-DOPA in treatment. Now it is suggested that another progressive disease of the 'extrapyramidal' motor system, Huntington's chorea, is associated with a specific deficiency of GABA (γ -amino-butyric acid) in the same brain regions.

GABA, known for 20 years to be present in brain, has a regional distribution which follows that of its synthetic enzyme glutamine acid decarboxylase (GAD), and both are present in synaptic fragments. GABA is in high concentration within the corpus striatum, globus pallidus and substantia nigra, structures within the extrapyramidal motor system, and also within the cerebellar cortex and nuclei (Fahn and Coté, *J. Neurochem.*, **15**, 209-213; 1968). The presence of a high-affinity uptake system and the demonstration of release following neural stimulation provide further evidence that this substance has a neurotransmitter role, and microionophoretic data suggest that at several sites GABA is an inhibitory transmitter. The recent development of an immunohistochemical technique by Saito *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 269-273; 1974) for GAD may greatly facilitate the task of localising particular gabaminergic systems.

Huntington's chorea is transmitted as an autosomal dominant condition which may affect between 4 and 7 per 100,000 of the population. The disease owes its persistence, and its peculiarly sinister character, to the fact that the average age of onset (44 years, according to Wendt) is beyond the normal period of fertility. Thus by the time symptoms appear the patient may have

Optical systems for space



photograph by Tinsley

The mirror illustrated (made by Tinsley in Berkeley, California) is designed to be part of a rocket-launched optical system. The grid pattern is formed by a series of holes drilled through the core to minimise weight. Because of the high stresses experienced in a large object during a launch, the accuracy of machining is critical and is evident in the regularity of intersection of the holes.

children who in turn face the prospect that they may develop the condition. The disease has both neurological and psychiatric aspects in that the development of jerky, uncontrolled, choreiform movements precedes a steady progression into dementia. Panse (*Die Erbchorea*; Leipzig, 1942) has demonstrated that a substantial minority of cases pass through a paranoid psychotic phase with schizophrenia-like features early in the illness. An understanding of the mechanism of these changes might have implications beyond the field of motor control.

Perry *et al.* first reported a deficiency of GABA in the brains of patients dying from Huntington's chorea in 1973 (*New England J. Med.*, **288**, 337-342). Later that year Bird and his colleagues (*Lancet*, **i**, 1090-1092) and McGeer *et al.* (*Neurology*, **23**, 912-917) reported a reduction in the levels of GAD, which is presumed to be a marker for gabaminergic neurones. Now Bird and Iversen (*Brain*, **97**, 457-472; 1974) have presented a detailed study of the brains of 38 patients dying with the disease and 38 controls of similar age dying from other causes. In this study the time between death, post-mortem and subsequent freezing of the tissue was kept to a minimum. Glutamic acid decarboxylase activity in the choreic patients was reduced by 75% in the basal ganglia, but not in the frontal cortex. GABA concentrations were reduced by 50%, and choline acetyltransferase concentrations by 55%, although these latter were within the normal range in a substantial proportion of the brains. Tyrosine hydroxylase activities and dopamine concentrations did not distinguish the two groups although a subgroup with rigidity (a possible variant of the disease) had significantly higher dopamine concentrations. Bird and Iversen argue that the most consistent lesion in Huntington's chorea may be a loss of GABA-containing neurones, with, in some cases, a loss of cholinergic neurones. In a study on part of the same post-mortem material Hiley and Bird (*Brain Res.*, **80**, 355-358) have shown that there is sometimes a loss also of the cholinergic muscarinic receptor. That this post-synaptic change may precede the loss of the pre-synaptic cholinergic neurone is suggested by the observation that normal choline acetylase levels are sometimes present with reduced concentrations of the muscarinic receptor.

These findings provoke a number of questions concerning possible neurochemical-clinical correlations. For example although there is electrophysiological evidence for a role for GABA-containing neurones in the cerebral cortex, there is no evidence in the recent study that these neurones are affected by the disease process. Therefore on

the evidence thus far available the dementia of the disease must be explained on the basis of subcortical changes, or some non-gabaminergic cortical cellular dysfunction. Second, there has been considerable interest in GABA in the cerebellum (a paper by Wilkin and co-workers in a recent issue of *Nature* (**252**, 397; 1974) presents convincing radioautographic evidence that the inhibitory Golgi cell terminals on the granule cell dendrites are one cerebellar site at which a high affinity GABA-uptake system is present). Huntington's chorea is not generally considered to be a cerebellar disease, but there is sometimes histopathological evidence of cell loss in this structure. Is it possible that such cell loss, when it occurs, is also of GABA-containing cells? Finally one might speculate concerning the paranoid psychotic changes which are occasionally seen early in the disease. Recently there has been interest in the hypothesis that excess dopaminergic activity underlies the paranoid form of schizophrenia. As Bird and Iversen point out the histopathological atrophy in Huntington's Chorea may lead to a relative increase in the density of innervation by surviving nigro-striatal dopaminergic terminals. Therefore a neurohumoural imbalance at this level provides a possible basis for the development of a paranoid psychosis which in turn may disappear when continued cell loss leads to the supervention of dementia.

Superconducting flux structure probed with positive muons

from P. G. Harper

ONE of the characters making a brief appearance in the drama of parity non-conservation (1956) was the positive muon (μ^+). This fundamental particle is analogous to the positron, but differs in mass (about 130 times larger), and attendant decay neutrinos. It is emitted from the decaying positive pion (π^+) in a spin-polarised state, that is to say, with its magnetic moment tending to lie along its direction of propagation. The μ^+ is relatively long-lived with a half-life of 2.2 μ s, and decays by positron (e^+) emission. The angular distribution of the latter is anisotropic with respect to the direction of μ^+ polarisation.

These basic properties of the μ^+ enable it to be used as a probe to examine magnetic materials such as Fe and Ni, and very recently (*Phys. Rev. Lett.*, **33**, 969; 1974), the type II superconductive flux structure. The principle is that if a μ^+ rests in a local magnetic field, its magnetic moment (or spin axis) will rotate (precess) about

the field direction at a rate proportional to the field. Because of the emission anisotropy, the instantaneous axial tilt at the moment of decay imparts a corresponding perturbation to the e^+ angular distribution. The positron emission, detected by coincidence counters in some fixed direction, thus follows the μ^+ precession so that the overall time decay is modulated at the precession period. For a field of 500 G, this would be about 0.01 μ s. Since the gyromagnetic ratio for μ^+ is known with considerable accuracy, the local magnetic field can be readily deduced.

The method thus resembles the so-called 'time-dependent perturbed angular correlation' technique employed with radioactive species. The advantage of a μ^+ beam is that it does no damage and leaves no residual contamination. Of course, when the method is employed to probe a non-uniform field distribution, the e^+ count records a space-averaged precession so that the frequency spectrum of the modulation requires careful interpretation.

The superconductive flux structure mentioned above is perhaps the most bizarre magnetic ordering found in any low temperature solid state phase. Present physical understanding of this phenomenon is fairly complete, and is based on developments of the remarkable 1950 theory of Ginsberg and Landau. Whereas for a thick specimen of lead, say, in its superconductive state, a weak applied magnetic field may be completely internally extinguished within 0.1 μ m of a parallel surface, in a type II material such as a lead-indium alloy, a similar field can penetrate right across the interior. It succeeds however only by condensing regularly into quantised filamentary flux lines (or vortices) which run parallel to the external field; they form a two-dimensional hexagonal structure with a flux-line density equal to the ratio of total flux to the universal flux unit, hc/e . At the centre of each flux line, the material is actually in its normal state, so that the induction achieves its maximum (that is, external) value. Away from the centre the induction declines, while at the same time the degree of superconductivity increases. Near the mid-point of neighbouring centres the induction forms a saddle-point. A strong hope for the μ^+ technique is that it might reveal and measure the saddle-point local field.

Of course, other methods have been used to probe the flux distribution, notably NMR, neutron diffraction, molecular beams and decoration microscopy. The present experiment therefore is largely to test a fairly novel technique, though additionally, it reveals some features of μ^+ behaviour.

The alloy $Pb_{0.90}In_{0.10}$, and Nb were

examined for comparison in the normal state, with the field applied, and with same field in the superconductive state. The latter was prepared by (1) cooling in the steady field to the mixed state, and (2) cooling in zero field, and then switching on. The results for the alloy were encouraging. For procedure (1), the main Fourier component of the modulated count (corresponding to the frequency of the applied field) was observed to broaden upon cooling to 5.5 K; upon further cooling to 3 K, a bifurcation was resolved, and a satellite peak identified as arising from the saddle-point mentioned above. Procedure (2) however showed no such splitting, indicating that the structure was smeared out by the preparation. Less exciting results were found for the Nb. No splitting was observed with either (1) or (2), but the main (external field) line appeared with much larger amplitude.

The authors conclude their report with a discussion concerning the possible diffusion of the μ^+ . Far from being simply trapped at a favourable location the tunnelling rate between Nb sites is estimated as being at least 10^{12} s^{-1} . If the observed sharp line in Nb is attributed to motional narrowing (as occurs in NMR for example) a large diffusion constant of the order $10^{-2} \text{ cm}^2 \text{ s}^{-1}$ is needed. At present, actual diffusion mechanisms remain speculative and the authors plan further work to resolve them.

Comet chemistry

from M. K. Wallis

"The Study of Comets", The International Astronomical Union Colloquium No. 25, held from October 28 to November 1 at NASA's Goddard Space Flight Center in Maryland, was timed to take stock of new data from comet Kohoutek and also to precede critical decisions on a possible cometary space probe.

Most of the active comet researchers from the United States and Western Europe put in an appearance, but unfortunately, in spite of sponsorship from the IAU and the International Committee on Space Research, nobody from the strong Soviet and Czech groups attended.

Much interest was attracted by the reports of the new molecular emissions, revealed in the ultraviolet and radio spectra. The 1,304 and 1,657 Å multiplets have given data on atomic oxygen and carbon, reported P. D. Feldman of Johns Hopkins University. And the radio observations, reviewed by L. E. Snyder (University of Virginia) have revealed CH, OH, HCN and CH_3CN in comet Kohoutek and H_2O in comet

Bradfield. Quantitative interpretation is thwarted however, as all except the HCN emission is pumped. The repeated failure to detect the predicted formaldehyde (H_2CO) emission is also put down to pumping.

Surprisingly, there is also a new identification in the optical—of H_2O^+ —a joint effort with P. Benvenuti (Asiago Observatory, Italy) and P. Wehninger (University of Tel-Aviv) as G. Herbig of Lick Observatory related. There are numerous lines from this ion, from the head and from far in the tail. They can be found on old spectra and the ion is probably very common as cometary plasma tails frequently show up on red-sensitive photographic plates, which should exclude the normal CO^+ emissions.

C. Arpigny of Liège (Institut d'Astrophysique) reported progress in the interpretation of molecular spectra, in particular in modelling collisional changes to the relative populations and line strengths of the purely resonant fluorescent description. Applying this potentially powerful method to the CH bands has given a reasonable total density in comet Bennett, with temperature of the colliding agent in the range 4–800°K. Analyses of the extensive H-coma were reported by H. U. Keller of LASP (University of Colorado), who has made this topic his own. All observations give outflow velocity in the range 7–10 km, which may correspond to the as yet unknown surplus energy of OH photodissociation. But the distant Lyman- α profiles of comet Bennett, observed from OGO-5, require for their explanation an atomic H component of higher velocity, around 20 km s^{-1} , as would be expected from the photodissociation of H_2O .

Including the new identifications, A. H. Delsemme (University of Toledo) advocated the simple two-stage picture of the cometary coma: H_2O and certain unknown parent molecules expand radially outwards and dissociate into daughter radicals and atoms, which are subsequently destroyed by ionisation. He gave length scales for the hypothetical parents and observed daughters. But a paper by C. K. Kumar and R. J. Southall of Howard University reported widely different scales in various comets for the supposed parents of CN and C_2 . And a chemist, R. Oppenheimer of Harvard, emphasised in a spirited contribution that numerous ion-molecule and dissociation-recombination processes can proceed rapidly inside 10,000 km in the coma. Various complex reaction chains could occur, resulting in CH , CN, C_2 and, in principle, even H_2O ! The fundamental constituents of comets are still very much in doubt.

H. U. Schmidt of Munich (Max-Planck Institut für Physik und

Astrophysik) reviewed progress in the fluid description of plasma motion through the coma. He considered that magnetic stress from the distorted field of the solar wind would reduce the distance of the contact discontinuity dividing off the purely cometary plasma, but explanations of how structures were produced from this plasma and gave rise to the observed tail were still lacking. M. K. Wallis (University of Oxford) argued, however, that because of cooling and recombination processes, the 'contact discontinuity' would occur inside the 10,000 km collisional region and therefore have little significance.

Sequences of pictures of ion tails of various comets had been made into a film by K. Jockers (Sacramento Peak Observatory). This illustrated particularly that subsidiary systems of rays sometimes arise from condensations in the tail and that nearby condensations and wave features move at similar speeds, so would appear to be convected features in a general flow. Progress was being made in the theory of dust tails, reported Z. Sekanina (Smithsonian Astrophysical Observatory). The fact that the 'silicate signature' in the infrared emission is not present in the anti-tail proves the particles differ in size.

There was as ever speculation about processes of comet formation: A. Mendis (University of California, San Diego) suggested that they could be accreted on the time scale of 10^5 years in present-day meteor streams; F. L. Whipple (Smithsonian Astrophysical Observatory) wished to use the compression of a solar gale from the young sun, while B. Donn (NASA: Goddard Space Flight Center) reckoned comets might be formed in a dense cloud within the cluster from which the sun emerged.

As to the long discussed mission to a comet, a space probe to comet Encke in 1980 now has a reasonable chance, reported I. Rasool (NASA). With a 'slow fly-by at 8 km s^{-1} ', the nucleus could be photographed and the neutral coma and plasma coma and tail could be investigated.

It is aimed to publish both impromptu and scheduled talks, by courtesy of NASA, in what will surely be voluminous conference proceedings.

Correction

The source of the illustrations from the ceiling of the British Museum (Natural History) (*Nature*, 252, 193; 1974) was not acknowledged: they were reproduced from the Botany Leaflet No. 1 published by the British Museum (Natural History).

articles

Pupils of a talking parrot

Richard Gregory* & Prue Hopkins†

Chance observations on a talking parrot have led to an investigation which reveals a striking temporal relationship between pupillary constriction and learned utterances.

A FEMALE talking parrot of the species known in this country as Yellow Fronted Amazon, Panama variety (*Amazona ochrocephala panamensis*), about nine years old and owned for the last five by one of us, exhibits marked constriction of the pupils of the eyes when she talks. She has a repertoire of about twenty words, calls, whistles and various recognisable sounds which are frequently uttered—accompanied by sudden and large pupil contraction. Once noticed, this is unmistakable, at least in Seraphita.

Although normally the pupils remain of almost constant size, even over large changes of illumination, during utterances

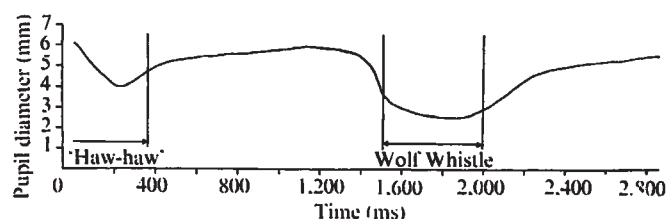


Fig. 1 Sample of analysed record (smoothed) showing shrinking pupil immediately preceding and during parrot's utterances.

they shrink rapidly to about half their resting diameter. This was such a clear and repeatable phenomenon that we decided to record it, and especially to discover time relations between the pupil shrinking and speech. During these investigations we found that the pupils would sometimes respond similarly while the parrot was listening to familiar words or sounds. Further, occasionally there would be a small shrinking of the pupils immediately prior to her speaking; as though, we thought, following some kind of internal 'rehearsal' for speech production. These further effects are smaller and more variable than the original effect observed, but after many hours of observation we are confident of them also, and have film records of their occurrence. It is known that the iris muscles are striated¹, which helps to explain the lability. There is no consensual reflex: varying illumination to one eye does not produce pupillary changes in the other eye. With the speech changes, however, both pupils move together.

The effects were recorded on several occasions, over a period of two years, on video tape with sound track. Technically satisfactory sections of the video tape records were transferred to 16-mm film, with magnetic sound strip. Transfer to film was undertaken because freezing the video tape on the video tape recorder (1-inch Ampex) gave an unsteady picture and

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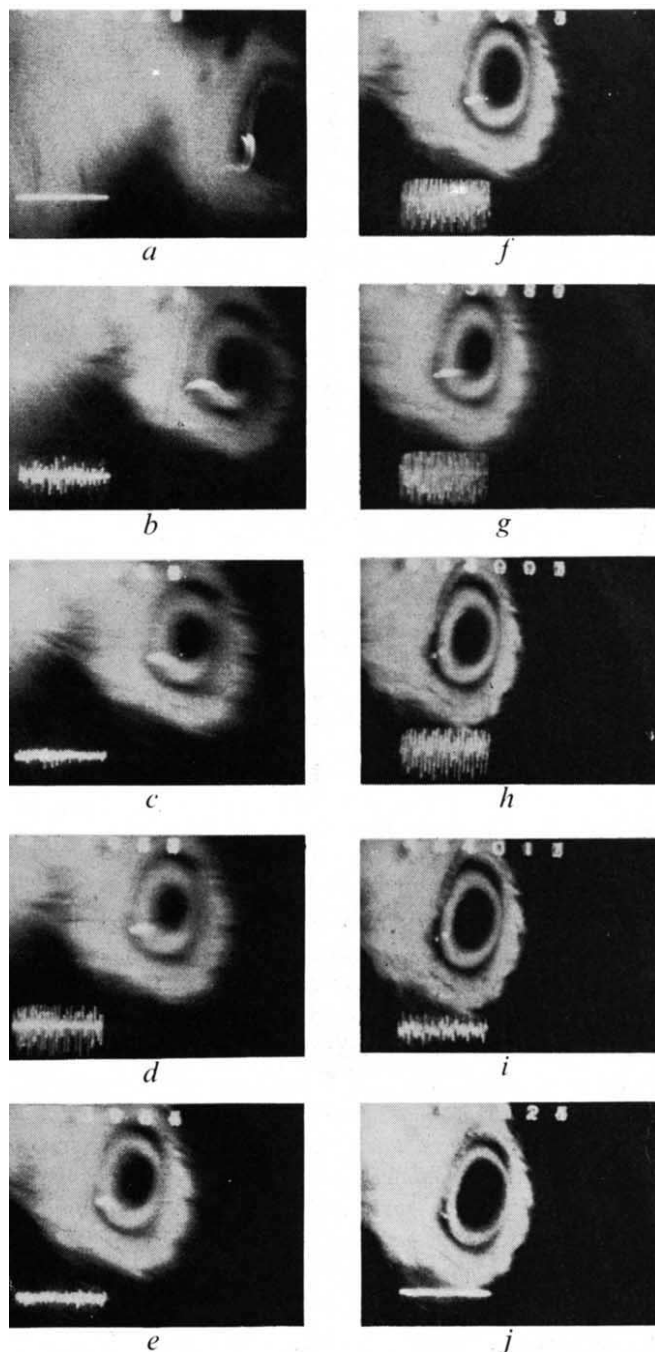


Fig. 2 Sequence from cine film of video tape, with oscilloscope record of parrot's 'wa-ha-he-e-y', electronically mixed in real time. a, Time 0; b, 240 ms; c, 400 ms; d, 480 ms; e, 720 ms; f, 1,000 ms; g, 1,240 ms; h, 1,520 ms; i, 1,760 ms; j, 2,080 ms. Speech started at 160 ms.

caused tape damage. The transfer allowed us to superimpose an oscilloscope record of the sound track, for measuring time relations between speech and pupil changes. The film camera was synchronised at 25 frames s^{-1} , to the 25 s^{-1} frame scan of the video system allowing time intervals to be estimated in 40 ms steps. The stop-motion projector has no sound head, so each section of speech was first classified, on an editor with a magnetic sound head (whose viewing screen was however too small for analysis) and a tape cassette of the sound track was also made for later checks. The moving inner edge of the iris—the pupil—and the unchanging outer edge of the iris of a single eye, were traced frame by frame from the magnified image given by the stop-motion projector, for sequences selected for maximum definition of the eye and when the bird's head was still. Diameters of the outer edge of the iris and of the pupil were measured both horizontally and vertically. Pupil measures were only accepted when the measures of the outer edge remained essentially constant, when measured changes of pupil size could not be due to changes in distance of the eye from the television camera.

Pupil contraction was found to start 4–5 frames before the bird's speech, which was clearly shown by the superimposed oscilloscope record of the sound track. The pupil contraction is therefore anticipatory to its speech by about 200 ms. Often contraction is nearly completed at the start of the utterance. The pupil diameter, both horizontal and vertical, shrinks by up to half the resting diameter. Contraction seems to occur for all utterances except for (innate?) very short squawks, present on sound tracks only for single frames of the film and so for less than 40 ms duration. Contractions are shown in the graph (Fig. 1) and in the sequence of cine frames from video tape (Fig. 2).

Pupil changes associated with our utterances were more variable, and by no means always present. They seemed to occur only when the parrot was 'attending'—gazing at us with a single steady eye—and only when we uttered one of her favoured words or sounds. Some of these are her name, "Seraphita"; "Hello"; "Goodbye"; "Good boy"; "Arrio" (a Spanish exclamation);

"Pretty boy"; "Prue"; and other names of her intimate acquaintance. She also has a very human laugh and an accurate telephone-bell sound. The pupils contract most dramatically for what can only be described as a 'wolf whistle'. All these are uttered spontaneously, and they may be evoked by saying them to her. Most recently, we find that she can develop a new call within seconds of hearing a new sound. She produced an accurate copy of the distinctive whir of a motor-driven Nikon camera, immediately after its first brief run, when we were trying to capture her pupil closure with high resolution directly on film. This 'Nikon whir' call was spontaneously repeated several times.

A younger male blue-fronted Amazon (*Amazona aestiva*) was introduced into a neighbouring cage a year ago. This parrot does not talk. His pupils are found to remain of constant size—except occasionally when he picks up an unfamiliar object when a small contraction may take place. This has also occasionally been observed for Seraphita. So pupil contraction seems to be mainly though not quite exclusively associated with utterance, attending to certain familiar utterances and sounds and also sometimes, we are inclined to believe, to internal 'rehearsal'.

We have so far no theory to account for these phenomena; neither do we know how general they may be among talking birds. Could they be signals to command other birds' attention? The contraction of the jet black pupils surrounded by their brilliant gold irises is distinct enough for this. Are they associated with difficult tasks, including handling unfamiliar objects, when depth of field may be increased and perhaps also the lens accommodated for near precise vision? Or is this phenomenon no more than (in two senses?) a case of neural cross talk?

We conclude that talking parrots can be and have apt pupils—but we cannot guess what they are apt to do.

We thank Philip Clark, Tony Makepeace, Anthony Downing and Freja Gregory for technical assistance.

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¹ Walls, G. L., *The Vertebrate Eye and its Adaptive Radiation*, 645–647 (Hafner, New York and London, 1963).

Audiofrequency vibrations and gravity-wave detectors

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Non-elastic audiofrequency vibrational modes in aluminium and other metals are suggested as a possible reason for the differences reported by various observers using large aluminium alloy bars (or disks) as gravity-wave detectors. Loading of suspended gravity-wave detector bars resulting from the gravitational attraction of the Earth is also sufficient to produce the small stresses required for the self-generation of audiofrequency sounds (acoustic emission) in materials. Both the non-elastic mode response and acoustic emission frequencies depend critically on the exact microstructure and/or residual stress state of a bar.

GRAVITY-wave detectors dependent on the excitation of elastic-wave vibrational modes in a solid have been cited variously as confirming^{1,2} or not confirming^{3–6} the existence of gravity waves. Most of these detectors consist of long bars of an aluminium alloy (exact alloy often unspecified) with

lengths chosen to give the first or fundamental longitudinal elastic mode of vibration in the audiofrequency range. The longitudinal modes of elastic vibrations in a free-free bar or rod have frequencies⁷,

$$v_n(\text{elastic}) = nc_l/2l = (n/2l)(E/\rho)^{1/2} \dots \quad (1)$$

where $n = 1, 2, 3 \dots$, l is the length of the rod, c_l is the longitudinal sound velocity in the rod, E is the longitudinal (Young's) modulus of the rod, and ρ is the density of the rod material. Longitudinal sound velocities in aluminium alloys⁸ range from about 4.5 to 6.4×10^5 cm s^{-1} so that rod lengths of 153 and 366 cm have been used for detectors with first-mode frequencies ($n = 1$) of 1,661 and 710 Hz, for example^{1,2,5}.

There are also non-elastic audiofrequency vibrational modes which depend critically on the exact microstructure or "texture" of a given sample, but are independent of elastic-wave velocities and macroscopic sample dimensions^{10,11}. That is, non-elastic mode frequencies, v_{kg} , corresponding to an ordered arrangement of atomic masses, m_k , with spacings, d_k , extending over a

distance, $S = Nd_k$, in the material are given to a good approximation by

$$v_{kq}(\text{non-elastic}) \simeq (h/8m_k S^2) q^2 \quad (2)$$

where h is Planck's constant, and $q = 1, 2, 3 \dots (N-1)$. For these non-elastic modes to be observed in small-amplitude vibration experiments it is also necessary that some residual or applied steady stresses be present in the material to activate the modes. Characteristic lengths, S , are determined by the mosaic patterns in single crystals and the grain or subgrain diameters in polycrystalline materials. Such lengths are most often in the micrometre range¹².

Vibrations in aluminium

For aluminium a value of $S = 1.07 \times 10^{-4}$ cm gives a first-mode frequency of 1,660 Hz from equation (2); $S = 2.14 \times 10^{-4}$ cm corresponds to a first-mode frequency of 415 Hz and a second-mode frequency of 1,660 Hz. That result is particularly interesting since Hiedenreich¹³ has used electron-microscope transmission to show that pure polycrystalline aluminium, heavily worked at room temperature, consists of slightly misoriented domains about 2×10^{-4} cm across. Beck and Hiu¹⁴ confirmed these results, and Kellar, Hirsch and Thorp¹⁵ used an X-ray microbeam method to show that pure aluminium worked at room temperature first had domains about 3.6×10^{-4} cm wide which decreased to 2.2×10^{-4} cm after prolonged resting at room temperature. The low order non-elastic mode frequencies given by equation (2) are very sensitive to the exact value of S ; values of S , in turn, vary according to the thermal and stress history (quenching, cold-working, annealing, and so on) and states of a particular piece of a given material.

From equations (1) and (2) we see that identical mode frequencies for the two types of wave correspond to vastly different wavelengths. An elastic first-mode frequency of 1,660 Hz in aluminium corresponds to a wavelength of about 300 cm and the first non-elastic mode at the same frequency has a wavelength of 4.28×10^{-4} cm. Thus the usual piezoelectric crystal vibration detectors respond to the acceleration of some portion of a sample surface with dimensions much less than a wavelength (elastic mode) or much greater than a wavelength (non-elastic mode) at low audiofrequencies. In addition elastic mode frequencies for a bar of fixed length, l , increase as 1,2,3,4... whereas non-elastic mode frequencies for a given microstructural length, S , increase as 1,4,9,16...; a particular bar has only one macroscopic length, but may have a microstructure with several predominant characteristic lengths or a distribution of lengths about some mean value.

Experimental evidence of non-elastic audiofrequency modes (at room temperatures) for dynamic shear compliance measurements on small metal disks was first reported¹⁶ in 1957. Some of the differences in mechanical vibrational spectra found in samples formed from the same 0.25-inch diameter extruded bar of 2S aluminium are shown in Fig. 1 where the variation of complex shear compliance, $J^* = J' - iJ''$, with applied vibration frequency from 100 to 2,000 Hz is adduced for three samples. All sample disks were cut from the same bar and were of virtually identical dimensions. The first sample disks (a) have a large resonance near 1,040 Hz (actually a triplet with mode frequencies 1,040, 1,060, and 1,070 Hz). Smaller resonances appear at 1,355, 1,655, and 1,860 Hz. Other resonances, not shown in Fig. 1, were observed at 2,010, 2,370 and 2,520 Hz; 2,930, 3,260, and 3,480 Hz. The groups of triplet resonances are roughly in the ratios 16:25:36:49 so that first, second and third-order modes near 66, 265, and 595 Hz might be inferred; none of these, however, appears in the data for this first sample. The spectrum of the second set of disks (Fig. 1b) is quite different, with only small resonances at 250 and 970 Hz. The third sample (Fig. 1c) again displays a very small resonance at 265 Hz, a triplet at 970, 1,000 and 1,050 Hz, but no other dispersions below 2,000 Hz.

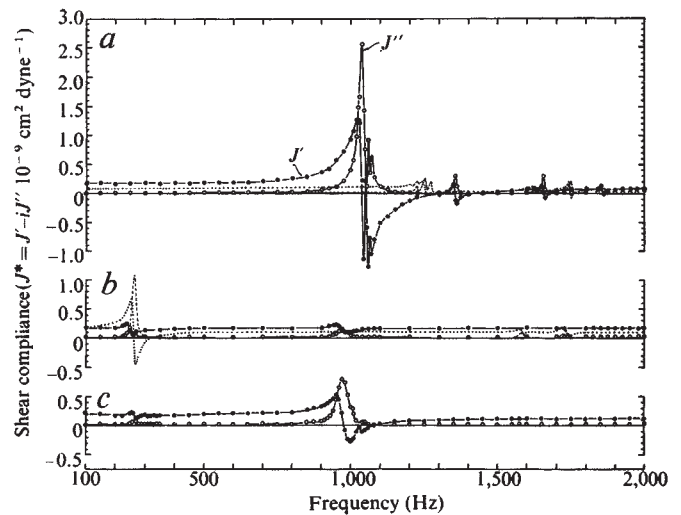


Fig. 1 Variation of complex shear compliance ($J^* = J' - iJ''$) with applied vibration frequency for three sets of small sample disks of 2S aluminium cut from the same extruded bar and of identical dimensions as described in the text. Dotted and dashed lines show measured values of J' and J'' respectively after No. 1 samples (a) were annealed at 1,100° F for two hours and after No. 2 samples (b) were compressed 3.0% in the direction of their axes. J' is the ratio of the in-phase component of resulting strain amplitude to the applied vibrating stress amplitude (a'/s_0) while J'' is the ratio of the 90° phase component of strain amplitude to the stress amplitude (a''/s_0) at each frequency. All measurements were made at room temperature ($24 \pm 1^\circ$ C) using the method and measuring apparatus described in ref. 17, and extended from March 15 to May 14, 1962. Differences in measured compliances are attributed to variations in sample microstructures and stress states as discussed in text.

Cause of observed variations

These differences are, of course, attributed to differences in microstructure and/or residual stresses which vary from place to place in a material. Much larger differences in microstructures and stresses are to be expected between (and within) large bars from 5 to 10 feet long and weighing thousands of pounds such as those being used for gravity-wave detection.

Thermal and mechanical stress effects on the shear compliance spectra are demonstrated by the dotted and dashed lines (for J' and J'' , respectively) in the groups of curves a and b in Fig. 1. These lines for the first sample (a) represent the results found after the sample was removed, annealed at 1,100° F for 2 h, found to have the original dimensions within ± 0.0001 inch, and then replaced in the measuring apparatus. Resonances above 2,000 Hz except the one at 2,920 Hz (moved to 2,930 Hz) also disappeared from the spectrum of this sample after annealing. By contrast the lines for the second sample (Fig. 1b) show the results found after this sample was removed and subjected to a 1,000-pound compressive stress on its parallel faces for one minute. The (mean) thickness decreased from 0.200 to 0.194 inch (3.0%) and the diameter increased as a result of this loading (major axis 0.2558, minor axis 0.2555). In addition to the large increase in the resonance at 260 Hz and the other changes shown in Fig. 1, the large resonance at 2,910 Hz 'moved' to 3,090 Hz after this load treatment.

The effects of annealing on the mechanical spectrum of the first set of 2S aluminium samples (Fig. 1a) are entirely similar to those previously reported¹⁶ for lead disks (99.9998% purity).

Relevance to 'gravity-wave' observations

This discussion of the occurrence of non-elastic vibrational modes in materials is presented as a possible explanation for the differences found among various gravity-wave detectors; a particular aluminium alloy bar designed to be sensitive to vibrations at an elastic-mode frequency may, in fact, be responsive to an intrinsic, non-elastic microstructural mode

at the same (or nearly the same) frequency. Another bar of the same chemical composition and dimensions, even if cast or formed under supposedly identical conditions, may, because of slightly different thermal and/or stress histories or states, have a different microstructure and/or (mean) residual stress state which make it less responsive to the non-elastic vibrational mode frequency or frequencies found in the first bar.

A second possible explanation of the differences is that stress-induced acoustic emission (at particular, discrete frequencies) could be present and detected in some of the bars, but not in others. The same wave-mechanical theory of deformation^{10,11} which describes the non-elastic vibrational modes previously cited also predicts the occurrence of small-amplitude audiofrequency vibrations during plastic deformation. An approximate expression for the net translational oscillating force on a lattice segment of length S in a material during steady loading is given by¹¹

$$f_q(t) \simeq (B_1 h / S d_k) q \exp(-i 2 \pi \nu_{k,q} t) \quad (3)$$

where h is Planck's constant, d_k is the regular spacing between atoms of mass m_k , extending over a distance $S = N d_k$, B_1 is a velocity constant depending on the load magnitude (the macroscopic stress) and the oscillation frequency, $\nu_{k,q}$, is given by equation (2), where $q = 1, 3, 5, \dots$. An estimate¹¹ of the mean force amplitude in equation (1) for $q = 1$ and $B_1 = 10^8 \text{ cm s}^{-1}$ is about $2 \times 10^{-8} \text{ dyne cm}^{-1}$. An oscillating force on the individual ordered segments of micrometre length within materials will tend to set into vibration (portions of) the entire sample; these sample vibrations will ordinarily be of very small amplitude unless a number of individual segment vibrations occur simultaneously throughout some appreciable volume of the sample. Contributions from even modes are absent from the net translational forces on segments ($q = 1, 3, 5, 7, \dots$, equation (3)), but even modes do produce net oscillating moments on the lattice segments¹¹ with amplitudes $(B_1 h / 2 d_k) q$ for $q = 2, 4, 6, \dots$.

The occurrence of audible sounds during severe plastic deformation is, of course, well known^{17,18}. Of more interest to the present discussion of gravity-wave detectors, however, is the occurrence of much fainter sounds at very low stress (and strain) levels, continuing through yield as reported by Kaiser¹⁹. Sensitive piezoelectric crystal detectors similar to those used on the gravity-wave detector bars, and a very quiet loading system, were used by Kaiser to study audiofrequency sounds emanating from polycrystalline zinc, steel, aluminium, copper, lead and wood specimens loaded in tension.

An extensive investigation of acoustic emission has been carried on since 1954 by Schofield²⁰⁻²² over a range of stress levels, and on both single crystals and polycrystals of gold, zinc, aluminium, lead, cast tin, carbon steel and 24ST-4 aluminium. A chief conclusion reached by Schofield is that acoustic emission consists of two distinct types: (1) random, low frequency, high amplitude bursts or pulses at low stresses; and (2) a continuous high frequency emission of initial low amplitude which increases in amplitude with increasing applied stress. Schofield also found that acoustic pulses are of extremely low energy, and amplifications of 10^6 to 10^7 are required for the detection of individual pulses. In some deformation processes avalanches of pulses may occur to produce a combined signal of much more energy.

Thus the possibility of acoustic emissions must be considered in connection with the large aluminium (or aluminium alloy) bars used for gravity-wave detectors. Many of these bars are suspended from their midpoints by steel cables or chains. Piezoelectric vibration detectors are usually placed around the bar circumference at the mid-point or sometimes on the ends. A distributed load resulting from the Earth's gravitational attraction acts on such a suspended bar of cross-sectional area A and length l with a value per unit length, $F/l = \rho g A$, where ρ is the density and g is the gravitational acceleration. Bending moments around the plane of suspension will also

be present, and at the cross-sectional plane of suspension a shear stress of $\rho g l$ exists. For a 2024-T4 aluminium bar of length 150 cm and diameter 19 cm, for example, this shear stress amounts to $4.06 \times 10^5 \text{ dyne cm}^{-2}$ (5.82 pounds inch⁻²). In addition, the bending moments will produce tension at the top of the suspension plane and compression at its bottom with mean stress values of around $1.5 \times 10^7 \text{ dyne cm}^{-2}$; exact calculations of the stress distribution can be made, but are not needed to demonstrate that the 'gravitational' stress levels in the bars are sufficient to produce acoustic emission at the rate of several pulses per second. Further, and of particular interest regarding gravity-wave detectors, Schofield observed in 1958 (refs 20-22):

"... The influence of the material from the microphysical standpoint appears to be a prominent factor determining the characteristics of the emission signals. Although the specimens were prepared from the same stock and are assumed to be 'identical' from the engineering viewpoint, the emission sources, if originating at the microlevel in the material, would be affected by the actual metallurgical and physical differences occurring locally..."

As suggested by equation (3) and confirmed by these experimental observations, bars of identical dimensions, chemical composition, and nominal structures may nevertheless 'emit' somewhat different audiofrequency vibrations under the same load because of differences in microstructures or initial stress states. In particular a narrow band or single-frequency vibration detector on one bar may record no signals because it is not tuned to any of the non-elastic mode frequencies corresponding to the characteristic microstructural length (or lengths), S , of that bar. A second bar, however, may have a non-elastic mode frequency identical to that of the detector frequency and display some of the audiofrequency bursts or 'events' which occur at very low stress levels.

Elaborate precautions taken to isolate the detector systems from ambient mechanical and sound vibration, shielding from electromagnetic radiation and so on, are all of little avail if possible vibrations generated within a detector bar are ignored.

Thus expectant observers of gravitational radiation should take into account their bar material microstates and the non-elastic vibration phenomena cited here.

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Palaeomagnetic evidence shows Malay Peninsula was not a part of Gondwanaland

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Palaeomagnetic results from Malaya show that the Malay Peninsula lay at 15° N during the late Palaeozoic, a result incompatible with the hypothesis that it once formed a part of Gondwanaland adjacent to India or Australia. Cretaceous results suggest the Peninsula was not at that time finally welded to the Asian mainland.

DEBATE has recently centred around the proposition that parts of South-east Asia were once a part of Gondwanaland. Burton¹ tried to explain the westerly origin of the sediments of north-west Malaya by supposing, in a reconstruction of Gondwanaland, that the west coast of the Malay Peninsula lay adjacent to

the east coast of India. His argument was supported by stratigraphic comparisons and the reasonably good fit of the coast-lines. Ridd² went one step further and fitted the whole of mainland South-east Asia and the Sunda shelf region between western Australia on the east, and the east coast of India on the west. This reconstruction was subsequently accepted by Audley-Charles *et al.*³ who incorporated it into their hypothesis of the development of eastern Indonesia. Stauffer and Gobbett⁴, however, raised serious objections to such a reconstruction from palaeoclimatic and other evidence. During the late Palaeozoic both India⁵ and Australia⁶ suffered continental glaciation and lay in high southern latitudes⁷. By contrast the

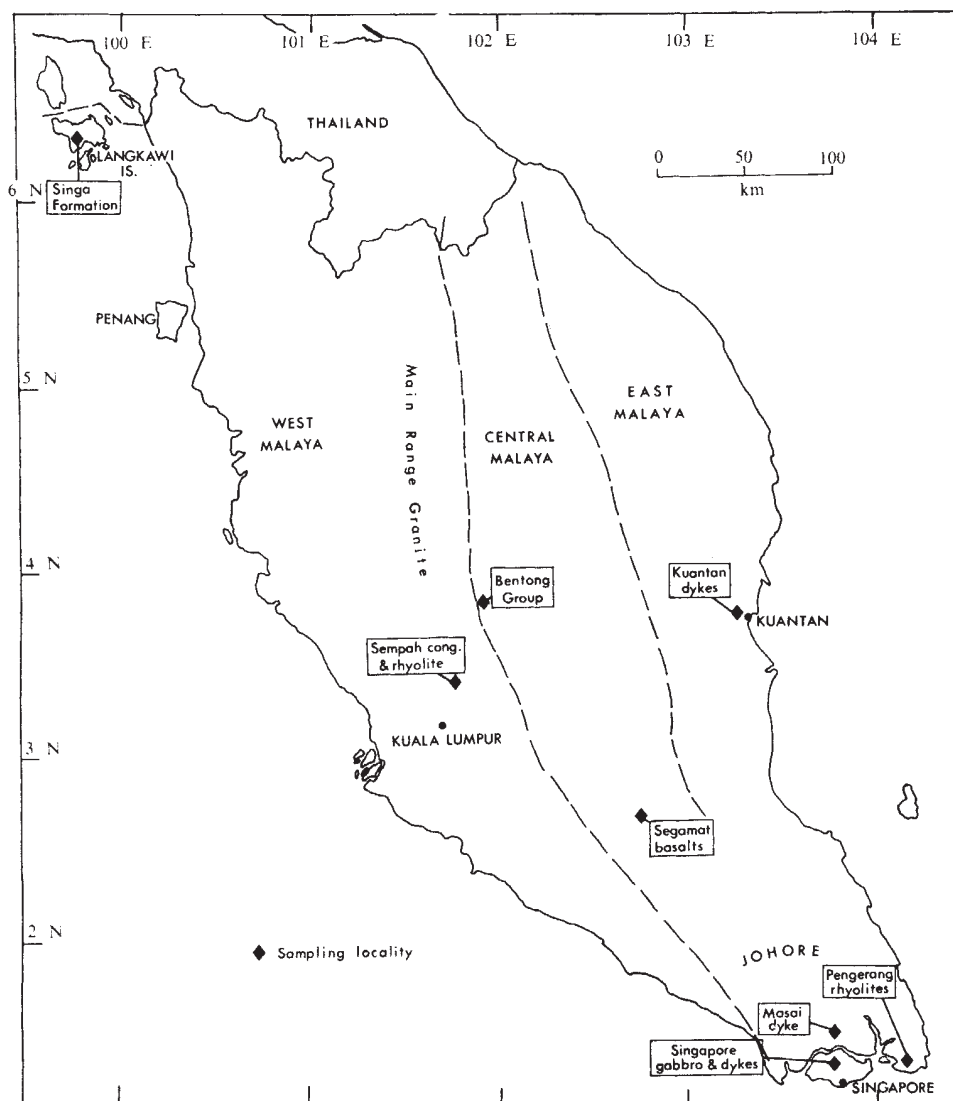


Fig. 1 Map of the Malay Peninsula showing sampling localities.

geological record of South-east Asia generally indicates tropical or subtropical conditions during the late Palaeozoic⁴. That the Permian of South-east Asia was laid down in warm water conditions is also accepted by Ridd⁸.

Both the palaeoclimatic problems and palaeogeographic reconstructions put forward can be tested using palaeomagnetic measurements. We report here the results of a reconnaissance survey of the palaeomagnetism of rocks of the Malay Peninsula. Some very preliminary results of this survey have already been reported⁹.

Geology and sampling

The Malay Peninsula can be divided into three geologically distinct zones shown in Fig. 1. Since the first discovery of Lower Palaeozoic fossils by Jones¹⁰ it has been found that Lower Palaeozoic rocks are restricted to the western zone. Recent work in Thailand^{11,12} has recognised the extension of this western zone of Lower Palaeozoic rocks through peninsular Thailand into north-west Thailand and eastern Burma and northwards into Yunnan in China. This long geosynclinal belt has been referred to by Burton¹¹ as the Yunnan–Malay geosyncline. In the Malay Peninsula the boundary with the Lower Palaeozoic belt is approximately marked by the so-called Bentong Line¹³

Table 1 Analytical data for four samples of Pengerang rhyolites (total rocks)

ANU no.	Rb (p.p.m.)	Sr (p.p.m.)	⁸⁷ Rb/ ⁸⁶ Sr	⁸⁷ Sr/ ⁸⁶ Sr
71-699	223	38	17.238	0.7692
71-700	250	35	20.584	0.7784
71-702	247	120	5.967	0.7302
71-703	224	134	4.8214	0.7273

The decay constant for ⁸⁷Rb used was $1.39 \times 10^{-11} \text{ yr}^{-1}$ with statistical parameters appropriate to the experimental precision of the instrumental procedures.

ANU, Australian National University.

which includes abundant radiolarian chert, some serpentine masses, metabasites and dolerite sills. It has been suggested that this marks the former position of a late Palaeozoic subduction zone¹⁴. The central zone is characterised by folded clastic Triassic–Jurassic carbonate and continental deposits whilst the eastern zone is composed of predominantly deep-water marine clastic Upper Palaeozoic rocks with patches of Upper Mesozoic continental deposits. The central and eastern regions are together considered by Stauffer¹⁵ to be an island arc formed in late Palaeozoic times and welded on to the block to the west. The most recent summary of the geology of the Malay Peninsula

is given by Gobbett and Hutchison¹⁶. In the present study hand samples, oriented by Brunton compass, were collected from a number of sedimentary and volcanic formations and igneous intrusions ranging in age from Carboniferous to Cretaceous (Fig. 1).

Six samples of red muddy sandstone from the Bentong Group were collected between mileposts 75 and 76 on the road from Kuala Lumpur to Raub (3.8°N, 101.8°E). The dip of the beds ranges from 30° to 70°, and in this region they are overlain, apparently conformably, by fossiliferous Viséan shales and sandstones²⁷. The samples are thus probably Lower Carboniferous, although they could be older.

Three samples of laminated muddy sandstone were collected from the Singa Formation on the south-west part of Langkawi Island, off the north-west coast of the Malay Peninsula (6.2°N, 99.8°E). The formation covers the whole of the Carboniferous¹⁷ and the stratigraphic position of the samples suggests they lie in the upper part of the Lower Carboniferous. The beds have an easterly dip of between 25° and 30°.

Twelve samples of reddish sandy mudstone were collected from the Sempah conglomerate together with seven samples of the overlying rhyolite in the Genting Sempah area on the Ulu Kali Road (3.4°N, 101.8°E). The rocks dip east at angles between 20° and 65°. Although inconclusive, Rb–Sr dating of the rhyolites¹⁸ suggests they are most probably Early Permian in age with a maximum possible age of Late Carboniferous.

Ten samples were collected from rhyolites occurring at Pengerang in south-east Johore (1.4°N, 104.2°E). The lavas dip north-west at between 19° and 36°. Rb–Sr measurements on these samples suggest they are Early Triassic or Permo-Triassic in age. This is identical with the age of the south Johore and Singapore granites¹⁸, with which they are probably associated.

Ten samples of basalt and two of red agglomerate were collected from the Segamat basalts of Negri Sembilan (2.5°N, 102.8°E) described by Grubb¹⁹. The lavas lie unconformably on Middle to Upper Triassic rocks. The flows appear to dip in a south-westerly direction at 15°, although this dip may be in part original. A single, whole rock, K–Ar age determination from these basalts gives an age of 62 Myr (ref. 18) but this is regarded as a minimum age in view of demonstrated argon loss from other rocks in the region¹⁸. The basalts are probably Cretaceous in age.

Three samples were collected from basaltic dykes near Kuantan, Pahang (3.8°N, 103.3°E) and two from a dyke at Masai, Johore (1.5°N, 103.8°E). One of the Kuantan dykes gives a K–Ar age of 110 Myr and is therefore probably Cretaceous in age¹⁸.

One sample of gabbro and four samples, one from each of four thin dykes intruding the gabbro, were collected from two quarries on Singapore Island (1.3°N, 103.8°E). The gabbro is intruded by the Singapore granite of Early Triassic age¹⁸ and basic dykes are not known to cut the granite. The dykes are

Table 2 Mean directions of magnetisation observed from a reconnaissance survey of Malayan rocks

Rock unit	Age	<i>n</i>	<i>D</i>	<i>I</i>	<i>R</i>	<i>k</i>	α_{95}
Segamat basalts, Kuantan and Masai dykes	K	16	136	−31	15.081	16.3	9.4
Pengerang rhyolites	(Pu-)Tr1	10	206	−39	9.638	24.9	9.9
Sempah conglomerate and rhyolites	(Cu-)Pl	14	212	−32	13.083	14.1	10.9
Bentong Group and Singa formation	C(l)	8	213	−16	7.725	25.4	11.1
Singapore gabbro and dykes	> Tr1	5	194	+21	4.758	16.5	19.3

n, No. of samples; *D*, declination east of true north; *I*, inclination—positive downwards; *R*, resultant of the *n* unit vectors; *k*, precision parameter; α_{95} , radius of circle of 95% confidence about the mean.

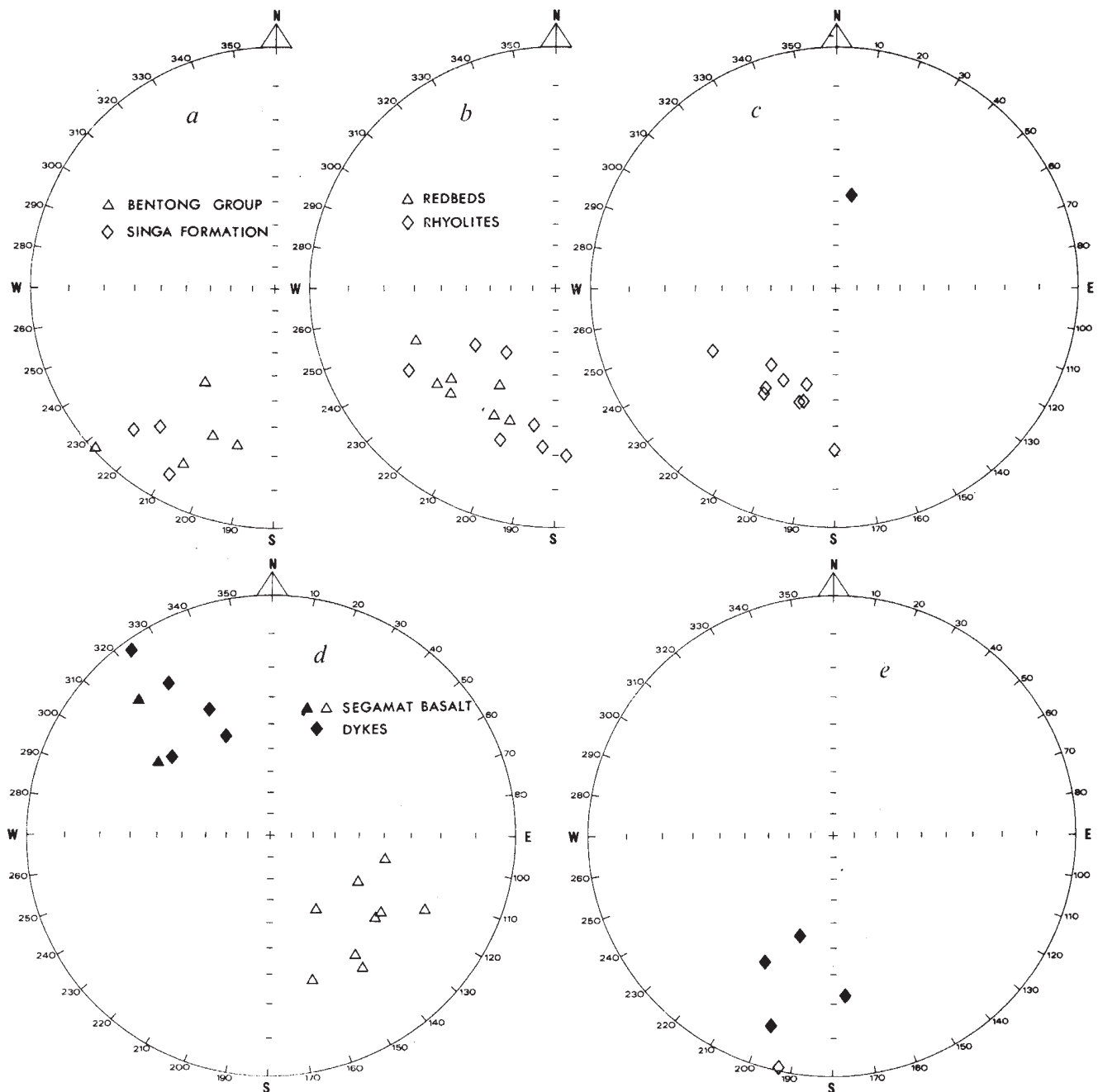


Fig. 2 Stereographic projections showing the cleaned directions of magnetisation for (a) Bentong Group and Singa Formation, (b) Sempah conglomerate and rhyolite, (c) Pengerang rhyolites (d) Segamat basalts, Kuantan and Masai dykes, (e) Singapore gabbro and dykes. Solid symbols on the lower hemisphere, open symbols on the upper hemisphere.

Several specimens were cored and sliced from each hand sample and the natural remanent magnetisation was measured using a PAR-SM1 magnetometer. Pilot specimens were subjected to alternating field demagnetisation in increasing fields²⁰. Most of these measurements were made at the Australian National University but during the final stages of this survey some additional measurements were made in the newly established palaeomagnetic laboratory in Kuala Lumpur. Measurements were then made on a Digico magnetometer and magnetic cleaning undertaken with a Schonsted demagnetiser. In Canberra, pilot specimens of the sedimentary samples were also subjected to stepwise thermal demagnetisation in which the specimens were heated and cooled in a magnetic field of one γ (1nT) or less²¹.

probably of similar age to the gabbro which must be older than Early Triassic, but by how much is unknown, as there is no evidence which could define a maximum age.

Rb-Sr age of the Pengerang rhyolites

Four samples of Pengerang rhyolites were analysed as total rock samples at the Australian National University by the Rb-Sr method (see Table 1). The individual analyses show inadequate enrichment in radiogenic strontium to make reliable estimates

of age. A statistical analysis using the least-squares method²⁵ with a two-error regression²⁶ indicates an age of 238 ± 23 Myr. The high mean square of weighted deviates (18.37) places this estimate in the category of a reconnaissance age determination, in so far as the samples probably had slightly different initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios; but the age can reliably be placed close to the Permo-Triassic boundary.

Bignell¹⁸ obtained an isochron of 225 ± 6 Myr for a combination of three analyses of Singapore granite and two of

Table 3 Statistical significance of the fold test applied to the four age groups using the table of McElhinny²²

Rock unit	Age	<i>n</i>	Directions			Poles		
			<i>k_b</i>	<i>k_a</i>	Sig.	<i>K_b</i>	<i>K_a</i>	Sig.
Segamat basalts, Kuantan and Masai dykes	K	16	18.5	16.3	No	22.3	18.6	No
Pengerang rhyolites	(Pu-)Tr1	10	13.9	24.9	No	9.4	21.5	Yes
Sempah conglomerate and rhyolites	(Cu-)P1	14	10.9	14.1	No	6.8	13.4	Yes
Bentong Group and Singa formation	C(1)	8	9.7	25.4	Yes	17.3	37.0	No

k_b, *K_b* are precision parameters before applying tilt corrections and *k_a*, *K_a* are those after applying tilt corrections.

Johore granites, using a decay constant of $1.47 \times 10^{-11} \text{ yr}^{-1}$. Converted to the 1.39 decay constant used in our analysis, this result gives a figure of 238 ± 6 Myr. Although this is also in the category of a reconnaissance result as the samples are from different localities, it is identical with that for the Pengerang rhyolites, which may provisionally be regarded as the effusive equivalent of the granites.

Palaeomagnetic results

Samples from the Bentong Group and Singa Formation responded well to thermal cleaning. Pilot specimens showed excellent stability up to the Curie Point of haematite. A small viscous component in the present field direction was removed at 300°C and on correcting for the dip of the strata, the samples group in a south-westerly direction with shallow inclinations (Fig. 2a). One sample was too weakly magnetised and its remanence could not be measured.

There was no difference in response of the red sediments from the Sempah conglomerate to either thermal or magnetic cleaning. Both the red sediments and rhyolite were therefore cleaned at 30 mT. Five of the twelve samples of red sediment, however, remained clustered about the present field direction, could not be cleaned and were rejected. This illustrates a problem associated with sampling red sediments in tropical countries. Even if samples are collected from road cuttings within a few years of exposure, weathering can have progressed fairly deep. In the case of red sediments it is sometimes very difficult to distinguish whether substantial deep weathering has occurred. Seven samples of red sandstone and seven rhyolites gave consistent directions after cleaning, similar to those given by the Bentong Group and Singa formations (Fig. 2b). All these late Palaeozoic samples are reverse magnetised.

The Pengerang rhyolites were extremely stable to alternating field demagnetisation, and blanket cleaning at 30 mT was carried out. One of the ten samples was normally magnetised and the other nine are reversed. The mean direction (Table 2, Fig. 2c) is very similar to that of the other late Palaeozoic groups. The basic rocks from Singapore and Johore and at Segamat and Kuantan were less stable than the rhyolites. One of

the Segamat basalts proved to be unstable but the remainder of the samples could be cleaned at 20 mT. The Segamat flows are all reverse magnetised whilst the red agglomerate and the dykes from Masai and Kuantan are normally magnetised

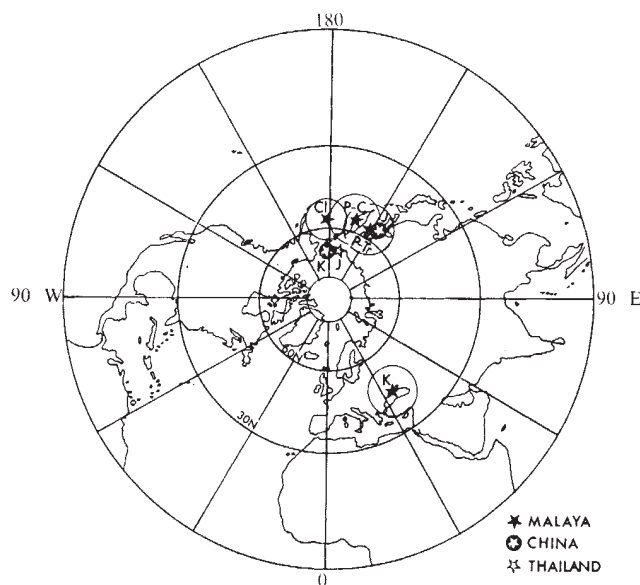


Fig. 3 Palaeomagnetic poles derived from the Malay Peninsula compared with those from China⁷ and Thailand²³.

(Fig. 2d). The Singapore gabbro and dykes are all reverse magnetised (Fig. 2e).

The mean direction of magnetisation for each of the groups is given in Table 2 together with the usual statistical parameters. The three late Palaeozoic means are very close to one another with only one of the 32 samples being normally magnetised.

Table 4 Palaeomagnetic pole positions calculated for the four age groups (mean of virtual geomagnetic poles)

Rock unit	Age	<i>n</i>	Lat. N	Long. E	<i>K</i>	<i>A₉₅</i>
Segamat basalts, Kuantan and Masai dykes	K	16	44	35	18.6	8.7
Pengerang rhyolites	(Pu-)Tr1	10	57	152	21.5	10.7
Sempah conglomerate and rhyolites	(Cu-)P1	14	55	164	13.4	11.2
Bentong Group and Singa formation	C(1)	8	57	182	37.0	9.2

n, No. of samples; *K*, precision parameter; *A₉₅*, radius of circle of 95% confidence about mean pole position.

Because of the different attitudes of the beds sampled, substantial tilt corrections have been applied. The statistical significance of the application of the fold test to four of the groups is given in Table 3 using the tables of McElhinny²². In spite of the relatively small collection of samples, there is a significant fold test for either the directions or poles for three of the four groups. This argues strongly for the stability of magnetisation of the samples at least since the time of folding, generally regarded as Late Triassic. The tilt corrections are too small for the Cretaceous group to produce any positive fold test. The presence of roughly opposed directions of magnetisation (Fig. 3d), however, provides additional stability evidence.

Palaeomagnetic poles and palaeogeography

Palaeomagnetic pole positions for four of the age groups are listed in Table 4. The five samples from Singapore are not considered sufficient to calculate a viable pole position and, in any case, the precise age of these rocks is not known. Between the Early Carboniferous and Early Triassic there appears to be a small westerly migration of the pole but it is hardly significant (Fig. 3). In fact, the data are such that it might be valid to combine all the 32 late Palaeozoic measurements together to

tudes of about 15°N during this time. This strongly supports the contention⁴ that the Malay Peninsula occupied low latitudes during the late Palaeozoic. Between the Permo-Triassic and the Cretaceous, the Peninsula underwent a clockwise rotation of 70° whilst maintaining much the same palaeolatitude.

The data for the late Palaeozoic may be used to test the hypothesis that the Malay Peninsula once formed a part of Gondwanaland. Since it is known that the late Palaeozoic is a period of predominantly reversed polarity⁷ the data of Table 2 indicate that the Peninsula lay 105° away from the South Pole, that is, in the northern hemisphere at 15°N. Using the latest estimate of the Permo-Carboniferous pole with respect to Gondwanaland²⁴, the Malay Peninsula must lie along a circle lying 105° away from this pole (Fig. 4). The palaeomagnetic data are clearly inconsistent with a position adjacent to India or Australia in the late Palaeozoic. Since Gondwanaland did not break up until the Jurassic, it seems unlikely that the Malay Peninsula was associated with the supercontinent in the early Palaeozoic and broke away before the other segments.

From Fig. 4 it can be seen that with due allowance for possible inaccuracies inherent in the method of positioning the Peninsula and Gondwanaland, the data do not completely rule out the hypothesis of Stauffer¹⁵ that the Malay Peninsula may have been adjacent to parts of north Africa in the Palaeozoic. Further palaeomagnetic work planned for the Lower Palaeozoic of the Malay Peninsula may throw more light on this hypothesis.

Samples from the Singa Formation were collected by Dr P. H. Stauffer. Discussions with Drs C. S. Hutchison, P. H. Stauffer and T. E. Yancey were helpful. D. Edwards and P. Schmidt assisted with some of the magnetic measurements.

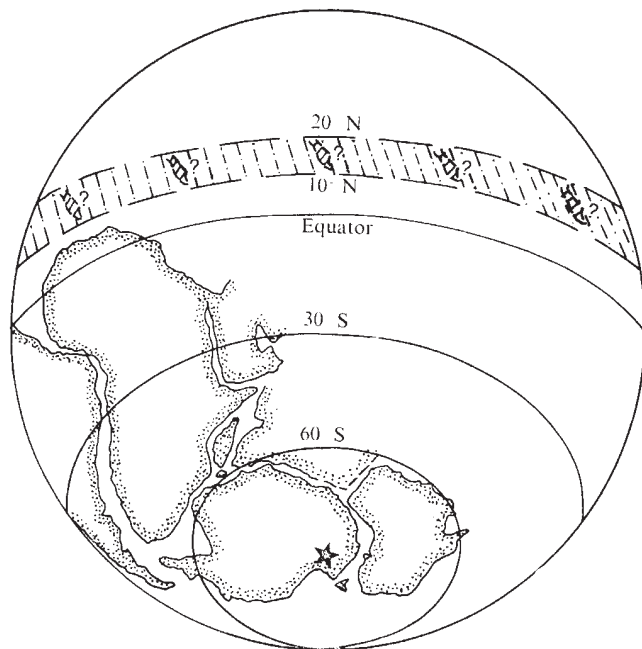


Fig. 4 Gondwanaland during the Permo-Carboniferous. The star is the mean palaeomagnetic south pole²⁴. The Malay Peninsula must lie at 15°N, anywhere along the arc of a circle 105° from the pole. A 10° wide belt is shown together with some possible positions of the Peninsula.

produce an overall pole at 56°N, 165°E with $A_{95} = 6.3^\circ$. The distribution of these poles changes from $K = 6.1$ to $K = 17.1$ after correcting for the attitude of the beds, a change that is statistically highly significant²².

The palaeomagnetic poles are plotted in Fig. 3 where they are compared with poles from the Jurassic of Thailand²³ and the Jurassic and Cretaceous of China⁷. These lie fairly close to the Malayan late Palaeozoic poles but are well removed from the Malayan Cretaceous pole. This would suggest that the Malay Peninsula was not firmly attached to the mainland in Cretaceous times. The magnetic inclinations observed during the late Palaeozoic and Cretaceous in Malaya are very similar (Table 2) indicating that the Peninsula continuously occupied low lati-

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Structural homology of myosin alkali light chains, troponin C and carp calcium binding protein

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Sequence comparison between the myosin alkali light chain, troponin C and carp calcium binding protein shows that the pattern of hydrophobic residues which forms the core of calcium binding protein is preserved in both troponin C and myosin alkali light chain. Strong structural similarities between the proteins are suggested. The role of gene duplication in the evolution of troponin C and myosin alkali light chain is discussed.

AN understanding of the mechanism of muscle contraction at the molecular level requires knowledge of the structure of the proteins involved and the ways in which they interact. The only muscle protein whose three-dimensional structure is known is a calcium binding protein from carp (CBP), often called parvalbumin^{1,2}. The function of CBP is unknown, but the amino acid sequence of the calcium binding component of rabbit troponin, troponin C (TNC)³ is closely related^{4,5}. Collins⁶ has observed a sequence homology between TNC and parts of the alkali light chains (ALC) of rabbit myosin^{7,8}. We have made a more searching comparison between these proteins using the completed light chain sequence⁹ to see how far the structural features of CBP are preserved in TNC and ALC. One aim was to try to discover why the free alkali light chains do not bind calcium and to account for the difference between the strong and weak calcium binding sites of TNC¹⁰. Another was to investigate the role of gene duplication in the evolution of these molecules¹¹. Finally the general interest in the role of light chains in the function of myosin makes these comparisons pertinent.

Structural considerations

The characteristic features of CBP, established from the X-ray diffraction studies of Kretsinger², may be summarised as follows. The protein contains two sites for binding calcium, related by a twofold symmetry axis. Each site lies in a corner between two helical regions, giving a basic structural unit; helix A-calcium site-helix B (abbreviated A-S-B). Duplication of this unit produces a two-unit structure (I-II) of the type A1-S1-B1-L-A2-S2-B2 (where L is a link region). CBP contains two additional helices at its N-terminal end and the packing of the six helices produces a hydrophobic core.

Figure 1a illustrates the structural features of carp CBP. Residues circled are buried in the core of the molecule² or make important hydrophobic contacts, and lines mark the main van der Waals' interactions. Figure 1a shows the pronounced asymmetry in the contacts between the four helices, such that the patterns of apolar groups are characteristically different. (In discussing these structures, a structural notation is used in which [A1(8)] refers to position 8 of helix A1.)

Ile [S(8)], (position 8 of the calcium site) is important structurally because of its buried side chain and a hydrogen bond linking the two adjacent Ile [S(8)] residues². Phe [S1(7)] may screen calcium site 1 from water, whereas site 2 has a lysine residue at [S2(7)] and may be more exposed. The amino acids which coordinate to calcium (Fig 1a) are listed in Table 1.

Important structural features in the link region are the salt bridge between Arg [L12(5)] and Glu [A2(3)], in a crevice between helices B1 and A2, and two apolar interactions between Leu [B1(6)] and the main chain of Arg [L12(5)], and between Leu [12(7)] and Thr [A2(4)]. The internal residues Leu [B1(8)] and Val [B2(8)] both appear to interact with the two N-terminal helices. If the structure of TNC reflects that of CBP, these positions might form part of the hydrophobic core in contact with the structural units III and IV.

Sequence comparisons

Comparison of TNC with CBP immediately suggested³ that troponin C contains four units of the type A-S-B. This structure could arise by further duplication of the calcium binding components of CBP to produce a tetrahedral molecule of the form: (A1-S1-B1-L12-A2-S2-B2)-J23-(A3-S3-B3-L34-A4-S4-B4), with a new joining piece J23. Using the complete sequence of the alkali 2 light chain⁹ we have aligned the sequences using a computer program which compared sequences span by span, with a span size of 11 residues¹². Similarities between amino acids were scored according to both their chemical similarity and the frequency of substitutions observed between them in a large number of different proteins. This procedure gives high scores for conservative replacements which fit readily into the same three-dimensional structure. The sequences of both TNC and ALC were compared with themselves as well as with each other, in order to identify internal repeats. Figure 1b and c shows the sequences drawn out to follow the structural features of CBP (compare Fig. 1a).

Table 1 Calcium coordinating groups in the calcium binding protein, troponin C and equivalent positions in the alkali light chains

Coordinating position	CBP		TNC				ALC			
	S ₁	S ₂	S ₁	S ₂	S ₃	S ₄	S ₁	S ₂	S ₃	S ₄
S ₍₁₎ X	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Gln
S ₍₃₎ Y	Asp	Asp	Asp	Asp	Asn	Asn	Thr	Gln	Glu	Asp
S ₍₆₎ Z	Ser	Asp	Gly	Ser	Asp	Asp	Asp	Asn	Gly	Asn
S ₍₇₎ -Y	Phe†	Lys†	Asp	Thr	Tyr	Arg	Lys	Lys	Val	Cys
S ₍₉₎ -X	Glu	Gly*	Ser	Asp	Asp	Asp	Thr	Glu	Met	Asn
B ₍₃₎ -Z	Glu	Glu	Glu	Glu	Glu	Glu	Gln	Gln	Glu	Ala
Negative charges	4	4	4	4	4	4	2	2	3	1

X, Y and Z refer to the octahedral vertices of the calcium ligating positions, with the sequence positions indicated.

*In CBP the Gly at position (-X) does not bind calcium, but the Asp at position (Y) links in both the Y and -X coordinating directions.

†In the (-Y) position, the carbonyl oxygen of the peptide bond coordinates to calcium.

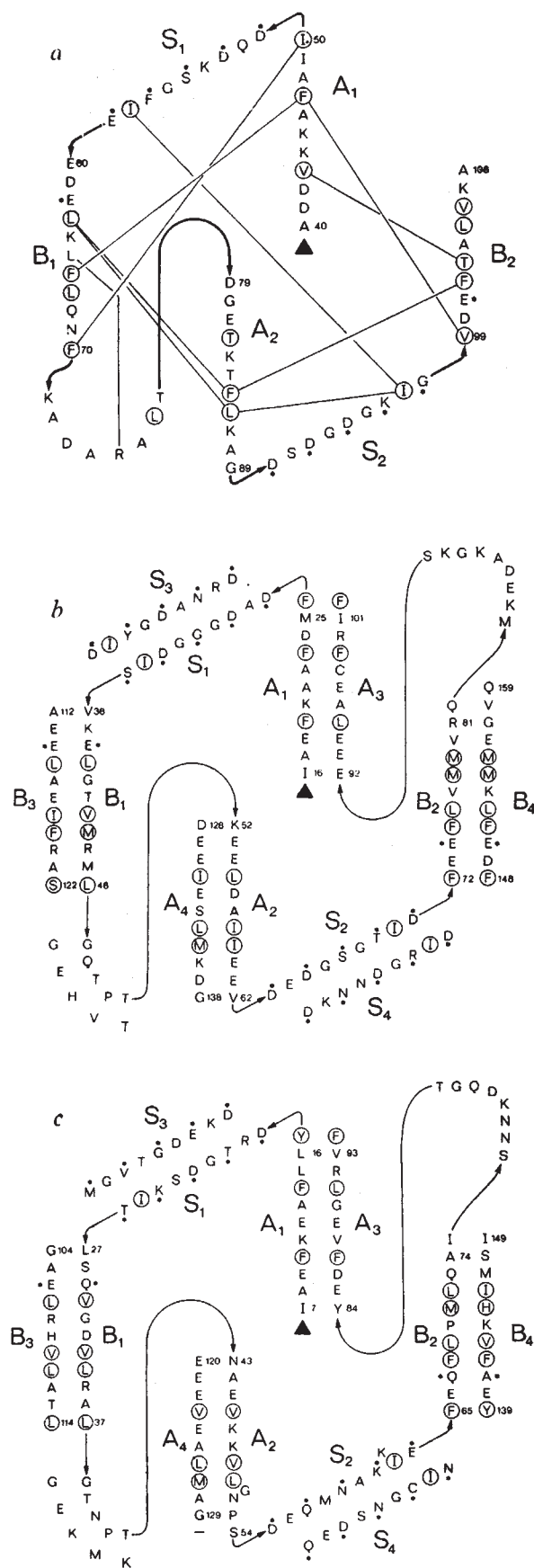


Fig. 1 *a*, Amino acid sequence of the two calcium binding structural units of carp calcium binding protein. Symbols A1, B1 and so on are the helical segments, while S1 and S2 denote the calcium binding sites. Residues circled are buried hydrophobic groups² or those making important hydrophobic contacts, and

The alignment of TNC with ALC is particularly good in the following regions: helix A1, the middle of helix B1 to L12 and the start of A2, A3, A4 and also the second half of S4 to the end of B4. As each sequence is repetitive there are also many cross-correlations, for example between TNC [L12] and ALC [L34] or *vice versa*.

The most striking observation is the preservation in both TNC and ALC of the pattern of hydrophobic residues which form the core of CBP. Table 2 summarises the data from Fig. 1, and shows that virtually every substitution is conservative. This suggests strong structural similarities between the three proteins. We have built a molecular model from Kretsinger's coordinates² to check that all the substitutions are feasible. Another residue which is conserved is the Glu at [A2(3)] and [A4(3)]. This is the position of the salt bridge in CBP, but the Arg in the link region is not present in either TNC or ALC. Link L34 of ALC contains two lysine residues, however, one of which might substitute in the salt bridge, while helices B1 and B3 of TNC and B1 of ALC contain arginine at position 9 which might preserve this bridge.

The only change required to fit both TNC and ALC to the CBP structure is to shorten the link region between helices B1 and A2, and B3 and A4. These links appear unnecessarily convoluted in the CBP model, and Pro [L12(4)] in both TNC and ALC could mark a bend which shortens this link. Also the glycine at the beginning of this region in both proteins could provide more flexibility at the start of the corner.

Internal repeats in TNC and ALC

Comparison of the sequence of TNC with itself shows clear evidence for internal repeats between structural units (I-II) and (III-IV), with unit I like III and II like IV, as observed by Collins *et al.*⁷. Helical regions which match particularly well are A2 with B4 (Fig. 1b). Such homologies are much stronger than those seen in normal proteins¹² and support the hypothesis of gene duplication in the evolution of this molecule. In ALC, the repeats follow a similar pattern but are much weaker. A surprising similarity which is not consistent with this pattern is the striking homology between A3 and B3 in TNC, which is analogous to that observed between helices A2 and B2 in CBP¹¹. Again there are similar homologies in ALC, between A2 and B3 and between A3 and B2.

Calcium binding sites

Each of the four calcium sites of TNC and both sites of CBP contain four acidic residues within the six coordinating positions, (Table 1). Comparison of the TNC sites shows striking similarities between S3 and S4 which contain six identical residues and conserve a basic residue at position

the lines mark the main van der Waals' interactions. *, Residues ligated to calcium ions; ▲, the start of the A1 helix is at residue 40 in the sequence, which follows the direction of the arrows to the C-terminal residue 108. For simplicity it has been assumed that each helix contains exactly 11 amino acids in every case. The one letter code for amino acids has been used in all the figures, where D=Asp, E=Glu, T=Thr, S=Ser, N=Asn, Q=Gln, K=Lys, R=Arg, H=His, G=Gly, A=Ala, V=Val, M=Met, I=Ile, L=Leu, Y=Tyr, F=Phe, P=Pro, C=Cys, and W=Try. *b*, Amino acid sequence of rabbit troponin C drawn out to correspond to the structural features of carp CBP, but with four calcium sites and four pairs of helices. ▲, The start of helix A1 corresponds to residue 16 in the sequence, while the C-terminus is at residue 159. *c*, Amino acid sequence of rabbit alkali light chain 2 drawn out as in *b*. ▲, The start of helix A1 corresponds to residue 7 in the sequence while the C-terminus is at residue 149. (The alkali light chain 1 has an additional 41 residues at its N-terminal end⁹.) Other than residues 7 and 8 at the beginning of helix A1, its sequence is identical to that shown.

S(2). The match between S1 and S2 is less good although five residues are identical.

These two matches between the pairs of calcium sites S1-S2 and S3-S4 form a different pattern from that predicted on the basis of gene duplication, that is the members of each pair are more alike than S1 to S3 or S2 to S4. When the sequence of CBP was aligned with those of TNC and ALC simultaneously, structural unit II of CBP showed close resemblance throughout to TNC (IV) and ALC (IV). This is also evident in the comparisons between the calcium coordinating residues of S2 in CBP and S4 in TNC (Table 1). The calcium sites show other quite strong similarities: the tyrosine in the -Y coordinating position of TNC [S3] is comparable with phenylalanine in CBP [S1]. S1 of TNC is like S2 of CBP in having no acid group at position -X, whereas the Asp at position Y binds to calcium in both the Y and -X coordinating position (Table 1). There are five identical residues in the comparison of these two sites, but TNC [S1] contains an aspartic acid at position -Y, which makes this site unique.

Isolated ALC does not bind calcium under physiologically significant conditions, and there are less than four negatively charged groups in every 'site' of this protein (Table 1). Examination of the molecular model indicates other changes which might reduce the affinity for calcium by distorting the binding sites. For example, helix A2 would be disrupted by the extra glycine residue following Leu [A2(8)] and proline at [A2(10)], while proline [B2(6)] would distort this helix also. Furthermore, site S2 has an extra basic residue inserted in front of Ile [S2(8)]. Similarly the presence of bulky valine and methionine residues could block site S3. Thus it is not surprising that ALC fails to bind calcium ions with high affinity even though many of the structural features of the sites are apparently preserved.

Considering the four calcium binding sites of TNC in more detail, Potter and Gergely¹⁰ have recently shown that isolated TNC contains two classes of calcium binding sites with different affinities. According to the CBP structural model, sites S3 and S4 are close together on either side of the approximate twofold axis at one end of the molecule, while sites S1 and S2 are close together at the other end. Sites S3 and S4 are most like the two sites of CBP, while S1 and S2 are more similar to one another than to either S3 or S4.

The most obvious interpretation of the sequences is that S3 and S4 have evolved into particularly strong binding sites, whereas S1 and S2 are weaker ones. But the structure suggests a more interesting possibility. There is evidence that the binding of calcium at the lower affinity sites of TNC switches 'on' muscle contraction and causes a conformational change in troponin (see ref. 13 for review). This suggests that during evolution the structure of the weaker binding sites acquired special constraints

which produced a lower affinity for calcium. The mechanism could then be similar to the binding of oxygen in haemoglobin, where the affinity of the haem groups is reduced by constraints in the tense deoxy structure¹⁴. On stereochemical grounds sites S1 and S2 which each contain two or more glycine residues, should be flexible and unconstrained with a high calcium affinity, while sites S3 and S4 would be more rigid and constrained. Also by analogy with CBP, site S3 with Tyr at S(7) is less exposed to solvent than site S4, which has Arg at S(7). Furthermore Site S4 has two basic groups at positions S(2) and S(7) whereas site S3 has only one. These facts suggest that site S4 may be the last to bind calcium and the first to release it, since the basic residues might weaken the calcium affinity of this site. It should be remembered, however, that the calcium affinities are altered in the complete troponin complex.

Gene duplication

The hypothesis that both TNC and ALC arose by two successive duplication events, producing first a dimeric structure and then a tetrameric one, is confirmed by Collins' analysis^{3,6}, and by our own computations. This is most clearly seen from the similarity between structural units I and III of TNC and between helices B2 and B4. The repeats are much stronger in TNC than ALC, suggesting that TNC is closer to the primitive sequence. CBP could then have evolved by adding two extra helices to the primitive dimeric unit¹ or by degradation of the tetrameric structure (I-II-III-IV) to (II-III-IV). The latter seems more likely since sites S1 and S2 of CBP closely resemble sites S3 and S4 of TNC, and the first helix of CBP is noticeably similar to the A helices of TNC. (The sequence of this helix is Asp-Ala-Asp-Ile-Ala-Ala-Ala-Leu-Glu-Ala-Cys.)

Proteins with a repeating sequence of the type X-X' and an approximate twofold axis are quite common, and probably often evolve from a structural dimer X:X. If an arbitrary structure X duplicates, it is highly improbable that the two halves of structure will fit together, and a good fit would require rapid evolution independently in the two halves leading to a structure X₁-X₂, with X₁ rather different from X₂. But if X evolves to form a well fitting dimer X:X before the duplication, the duplicated molecule X-X requires few modifications and the two halves can remain very similar. One suggestive example is the ferredoxin of *Peptococcus aerogenes*¹⁵, where the polypeptide chain has two repeat units and there are two similar iron binding sites formed by clusters of sulphhydryl groups. Surprisingly each site is formed by groups from both halves of the sequence, so that dimer formation is essential for iron binding to occur. It is therefore probable that the half-chain units evolved the power to interact before duplication produced a 'single chain dimer'. Applying this principle to TNC leads to the suggestion that a primitive unstable

Table 2 Pattern of hydrophobic residues in the A and B helices of CBP, TNC and ALC

Helix residue no.	Helix A1			Helix A3		Helix B1			Helix B3	
	CBP	TNC	ALC	TNC	ALC	CBP	TNC	ALC	TNC	ALC
4	Val	Phe	Phe	Leu	Phe	Leu	Leu	Val	Leu	Leu
7	(Ala)	(Ala)	(Ala)	(Cys)	(Gly)	Phe	Val	Val	Ile	Val
8	Phe	Phe	Phe	Phe	Leu	Leu	Met	Leu	Phe	Leu
11	Ile	Phe	Tyr	Phe	Phe	Phe	Leu	Leu	(Ser)	Leu
	Helix A2			Helix A4		Helix B2			Helix B4	
	CBP	TNC	ALC	TNC	ALC	CBP	TNC	ALC	TNC	ALC
1	(Asp)	(Lys)	(Asp)	(Asp)	(Glu)	Val	Phe	Phe	Phe	Tyr
4	(Thr)	Leu	Val	Ile	Val	Phe	Phe	Phe	Phe	Phe
5	(Lys)	(Asp)	(Lys)	(Glu)	(Glu)	(Thr)	Leu	Leu	Leu	Val
7	Phe	Ile	Val	Leu	Leu	Leu	Met	Met	Met	(His)
8	Leu	Ile	Leu	Met	Met	Val	Met	Leu	Met	Ile

The hydrophobic residues shown are those which play an important part in holding the helices and calcium sites together in the CBP structure. Residues in brackets are not hydrophobic, and demonstrate the asymmetry of the patterns of hydrophobic groups in the different helices.

structure of the type A-S-B was originally stabilised by formation of a dimer, and gene duplication subsequently yielded the two-unit structure (A-S-B)-L-(A-S-B). The similarity between helices A and B which has already been noted could be accounted for if the (A-S-B) unit itself was formed from a single precursor helix H and a piece of polypeptide chain, C. Dimerisation of H-C to stabilise the helices, followed by gene duplication would lead to H-C-H-C, the precursor of (A-S-B).

Light chain function

If carp calcium binding protein, rabbit troponin C and the alkali light chains have similar three-dimensional structures, they may also have related functions. While the function of TNC is most clearly understood, the role of CBP remains obscure¹⁶. The presence of parvalbumins in skeletal muscles of higher vertebrates may indicate a calcium binding role directly related to muscle contraction¹⁷. There is no evidence to support any calcium binding role for the alkali light chains, and isolated light chains do not bind calcium in the concentration range 10^{-7} M to 10^{-5} M in the presence of 1 mM Mg^{2+} ions (J. Kendrick-Jones, personal communication). Thus in spite of the structural similarity, this protein may have lost an original function related to calcium binding and now possess an entirely different function, possibly connected with the ATPase activity of myosin. Experiments to demonstrate participation of these light chains in the ATPase activity of skeletal myosin^{18,19} have given variable results and cannot be regarded as conclusive. The reaction of the disulphide analogue of ATP with the single thiol group in the S4 corner of ALC is suggestive but not conclusive, since other thiols in the heavy chains are also labelled²⁰.

Interest in the effects of calcium on myosin has been increased by the discovery that calcium regulation in molluscan muscles depends on a specific light chain associated with the myosin 'heads' (the EDTA light chain of scallop myosin)²¹. Thus mammalian muscle appears to have evolved a different form of calcium regulation, though the discovery of species with dual regulation²² has led to the speculation that vertebrate muscle might also contain a myosin regulatory system. X-ray evidence suggests a direct effect of calcium on vertebrate myosin^{23,24} and biochemical studies have sought to confirm this^{25,26}. Kendrick-Jones has recently shown that of the three light chains present in rabbit myosin, one (the DTNB light chain) can substitute for the EDTA light chain of scallop myosin and restore calcium sensitivity to the desensitised myosin²⁷. Thus this light chain acts as an inhibitor to switch 'off' the scallop actomyosin ATPase when calcium is removed. This suggests that the DTNB light chain is analogous to troponin

I in the actin-linked regulatory system rather than troponin C. It is now important to establish whether the DTNB light chain has an equivalent inhibitory function in mammalian myosin and whether, in living muscle, the alkali light chains have any function related to calcium regulation.

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An SV40-induced initiation factor for protein synthesis concerned with the regulation of permissiveness

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An SV40-induced factor, which catalyses binding of late human adenovirus mRNA to ribosomes to form the 80S initiation complex for protein synthesis, is present in high salt extracts of ribosomes from the transformed simian cells.

THE defective step of human adenovirus growth in African green monkey kidney (AGMK) cells seems to reside in the inability of ribosomes to bind with late viral mRNA for capsid proteins to form polyribosomes, since certain species of late viral mRNA are absent in polyribosomes of the infected cells

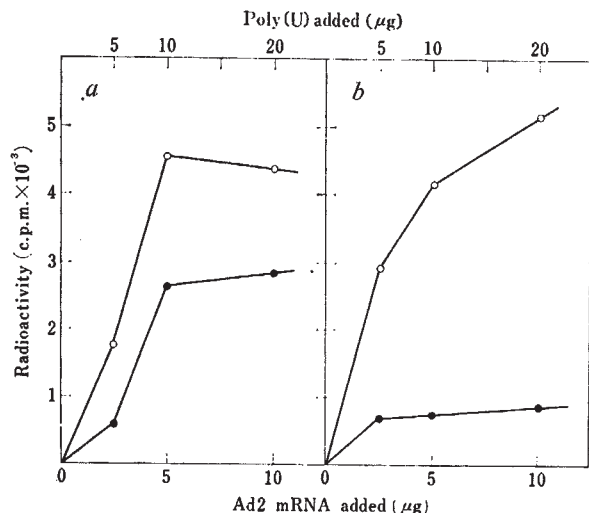


Fig. 1 Response of preincubated S30 to added RNA. The cells were washed with Earle's solution and suspended in 2 vols of TKM (10 mM Tris-HCl, pH 7.4, 10 mM KCl, 1.5 mM Mg(OAc)₂) at 0° C for 10 min. The swollen cells were disrupted by about 20 strokes of a Dounce homogeniser and the tonicity was immediately restored by the addition of 1/3 vol. of tenfold concentrated medium K (1 × medium K contains 30 mM Tris-HCl, pH 7.4, 120 mM KCl, 5 mM Mg(OAc)₂ and 3 mM dithiothreitol (DTT)). The homogenate was centrifuged at 1,000g for 5 min to remove nuclei and the supernatant was centrifuged at 30,000g for 10 min. The supernatant (S30) was supplemented with 5 μM each of 20 amino acids, 5 mM ATP, 0.5 mM GTP, 6 mM phosphoenolpyruvate (PEP), 10 μg ml⁻¹ of pyruvate kinase and incubated at 37° C for 50 min. The incubated S30 was centrifuged at 10,000g for 5 min and the supernatant was dialysed against medium K. The reaction mixture (0.15 ml) for amino acid incorporation contained 50 μl of preincubated S30, 1 mM ATP, 0.1 mM GTP, 10 mM PEP, 0.2 mg ml⁻¹ of pyruvate kinase, 20 μM of 19 naturally occurring L-amino acids (minus phenylalanine or leucine) and 1 μCi ml⁻¹ of ³H-phenylalanine (10 Ci mmol⁻¹) or ³H-leucine (32 Ci mmol⁻¹) in the presence of either poly(U) (○) or Ad2 mRNA (●). The mixture was incubated at 37° C for 1 h and the hot trichloroacetic acid (TCA)-insoluble count was measured. *a*, T22 cell S30; *b*, C14 cell S30.

although they are present in the cytoplasm¹. This defect, however, can be complemented either by coinfection or transformation of the cells with SV40, indicating that a factor(s) is induced by SV40 which might enable the ribosomes to bind with this species of late adenovirus mRNA.

Initiation of protein synthesis in *Escherichia coli* requires three factors (F1, F2 and F3)²⁻⁵ that associate with ribosomes, and similar factors (M1, M2 and M3)⁶⁻⁸ have been found in mammalian cells. Of these, F3 (M3) is concerned with mRNA selection by ribosomes and, therefore, has the regulatory function of deciding the species of mRNA to be translated. The function of this factor can easily be modified as has been demonstrated in *E. coli* after infection with phage T4 (ref. 9), T7 (refs 10 and 11) or MS2 (ref. 12).

Here we show that a factor(s) is present in the high salt extract of ribosomes from SV40-transformed AGMK cells which are permissive for human adenoviruses, but not in ribosomes from untransformed AGMK cells which are non-permissive. The factor enables ribosomes to bind with late adenovirus mRNA and to form the 80S initiation complex for protein synthesis. We discuss a mechanism of induction of this factor by SV40 in relation to the mechanism of cell transformation by oncogenic viruses.

Response of preincubated S30 to added RNA

Differential response to RNA of ribosomes prepared from SV40-transformed AGMK cells, clone T22 (ref. 13), and untransformed AGMK cells, clone C14 (ref. 13), was compared by protein synthesis with preincubated S30 in response to either late adenovirus type 2 (Ad2) mRNA or poly(U). Late Ad2

mRNA was prepared as poly(A)-containing RNA from polyribosomes of the infected KB cells as reported previously¹. The RNA was sedimented in an SDS-sucrose gradient and the 24S region was collected because the size of mRNA for hexon, a major component in adenovirus capsid proteins, was estimated as 24S on the basis of the molecular weight of hexon, 130,000 (ref. 14). A certain species of mRNA of this size is apparently absent from polyribosomes of the adenovirus-infected C14 cells but present in the infected T22 cells¹. As shown in Fig. 1, C14 cell S30 translates late Ad2 mRNA very poorly by comparison with T22 cell S30, whereas both translate poly(U) with almost the same efficiency. A slight incorporation of amino acids by C14 cell S30 in response to late Ad2 mRNA is probably caused by contamination of cellular mRNA, although poly(A)-containing RNA separated on poly(U)-Sephacrose consists predominantly of Ad2 mRNA as described previously¹. The gel electrophoretic analysis of the products (Fig. 2) showed that hexon was synthesised with T22 cell S30 in response to late Ad2 mRNA but not with C14 cell S30. These results indicate that a factor(s) responsible for translation of certain species of late Ad2 mRNA is present in SV40-transformed AGMK cells but not in untransformed AGMK cells.

Formation of 80S initiation complex

The presence of an initiation factor which catalyses binding of late Ad2 mRNA to ribosomes in the high salt extract of ribosomes (ribosomal wash) from SV40-transformed AGMK cells was demonstrated by formation of the 80S initiation

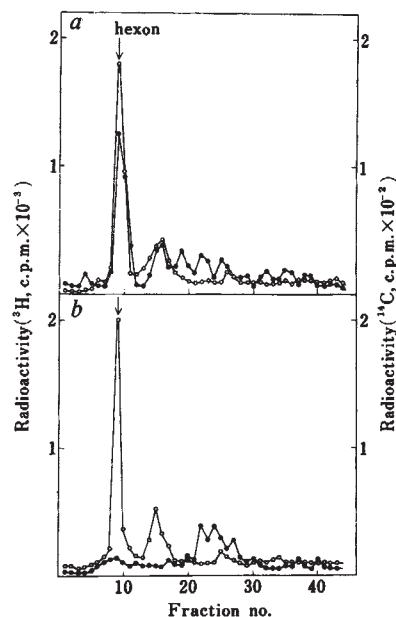


Fig. 2 Gel electrophoretic analysis of product synthesised with S30. Protein synthesis with S30 in response to late Ad2 mRNA was carried out as described in Fig. 1, except that ³H-leucine and 19 cold amino acids were replaced by ¹⁴C-amino acid mixture. After the reaction, pH was adjusted to 11.0 by the addition of 0.1 N NaOH and the mixture was incubated at 37° C for 15 min. The mixture was dialysed against 0.1 M sodium phosphate (pH 7.4) containing 0.1% SDS and 0.01% 2-mercaptoethanol overnight. The products were concentrated by evaporation at 37° C in dialysis tubing and Ad2 virions labelled with ³H-amino acids mixture were added. After heating at 100° C for 1 min, the products were subjected to electrophoresis. Electrophoresis was carried out on 7.5% polyacrylamide gel (10 × 0.6 cm) at 80 V (15 mA per gel) for 4 h. The buffer used was 0.05 M sodium phosphate (pH 7.2) containing 0.1% sodium dodecyl sulphate (SDS). Gels were then sliced and the slices were counted in 5 ml of Triton X-toluene scintillator after dissolution in 0.5 ml of 50% H₂O₂ at 80° C overnight. *a*, T22 S30; *b*, C14 S30. ●, ¹⁴C; ○, ³H.

complex for protein synthesis. Ribosomes washed with 0.5 M KCl were prepared from preincubated C14 cells S30. High salt extract of ribosomes was prepared from cells either permissive or non-permissive to adenoviruses. Met-tRNA_f was prepared by charging methionine to tRNA from T22 cells with *E. coli* S100.

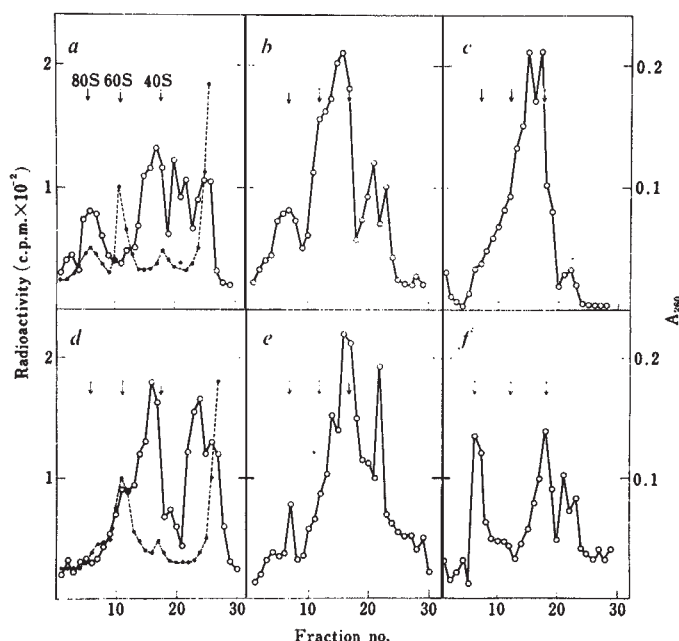


Fig. 3 Formation of the 80S initiation complex with Ad2 mRNA. Ribosomes were prepared from the preincubated C14 cell S30. The S30 was treated with sodium deoxycholate (0.8%) and centrifuged at 16,000g overnight through a cushion of 60% sucrose in medium K at 4°C. The pellet was suspended in 1 ml of 50 mM triethanolamine-HCl (TEA), pH 7.6, containing 0.5 M KCl, 1.5 mM Mg(OAc)₂ and 1 mM puromycin²² and incubated at 0°C for 10 min followed by incubation at 37°C for 10 min. It was centrifuged through a 10–30% (w/w) sucrose gradient in 50 mM TEA, pH 7.6, containing 0.5 M KCl and 5 mM Mg(OAc)₂ at 23,000 r.p.m. for 16 h at 4°C in a Spinco SW 25.1 rotor. The 40S and 60S ribosomal subunits, collected separately from the gradient, were stored at 0°C in medium K containing 20% glycerol after centrifugation at 16,000g for 5 h. Ribosomal wash fraction was prepared from crude ribosomes obtained from the preincubated S30. Ribosomes were gently stirred for 1 h at 0°C in 0.25 M sucrose containing 50 mM Tris-HCl, pH 7.4, 10 mM MgCl₂, 1 M KCl, 2 mM DTT, 10 mM 2-mercaptoethanol and centrifuged at 200,000g for 4 h at 4°C through a cushion of 0.5 M sucrose in the same solution. The top supernatant was dialysed overnight against 80% ammonium sulphate in 50 mM Tris-HCl, pH 7.4, containing 50 mM KCl, 0.1 mM EDTA, 10 mM 2-mercaptoethanol and 5% glycerol. The resulting precipitate, collected by centrifugation, was dissolved in 50 mM Tris-HCl, pH 7.5, containing 50 mM KCl, 0.5 mM DTT, 10 mM 2-mercaptoethanol, and 5% glycerol dialysed against the same buffer for 4 h at 0°C and stored at –80°C. Transfer RNA was prepared from T22 cells and amino-acylated by S100 extract of *E. coli*. Formation of the 80S initiation complex was carried out in four successive incubation steps according to Levin *et al.*¹⁵ The components added in each reaction step were dissolved in 40 mM Tris-HCl, pH 7.4, containing 1 mM DTT and 0.4 mM GTP. Each reaction was carried out at 37°C for 5 min. Step 1; (0.1 ml) 30 pmol of Met-tRNA, 75 µg of ribosomal wash proteins, 0.1 M KCl. Step 2; (0.1 ml) 0.25 A₂₆₀ units of 40S subunits, 80 mM KCl and 5 mM MgCl₂. Step 3; (0.05 ml) ³H-labelled late Ad2 mRNA (about 5,000 c.p.m.), 65 mM KCl and 5 mM MgCl₂. Step 4; (0.05 ml) 0.5 A₂₆₀ units of 60S subunits, 50 mM KCl, 5 mM MgCl₂. After the reaction, an aliquot of 0.3 ml was centrifuged through a 15–30% (w/w) sucrose gradient in 40 mM Tris-HCl, pH 7.4, containing 0.1 M KCl and 5 mM MgCl₂ at 48,000 r.p.m. for 100 min at 4°C in a Spinco SW 50.1 rotor. Each fraction was counted after cold TCA precipitation (○). Absorbance at 280 nm (●). Ribosomal wash proteins (75 µg) used were prepared from: a, T22 cells; b, KB cells; c, GC7 cells; d, C14 cells; e, Vero cells; f, T22 cells. Late Ad2 mRNA prepared from polyribosomes of the infected KB cells was used for a–e and that from the cytoplasm of the infected C14 cells for f.

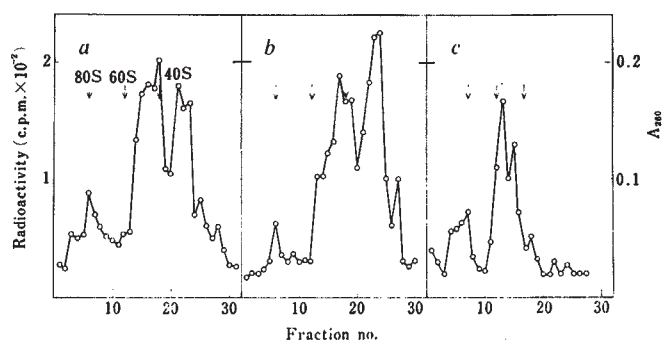


Fig. 4 Formation of the 80S initiation complex with SV40 mRNA. Formation of the initiation complex was carried out as described in Fig. 3. Ribosomal wash proteins (75 µg) used were prepared from: a, T22 cells; b, C14 cells; c, GC7 cells. SV40 mRNA was prepared as poly(A)-containing RNA from polyribosomes of the infected GC7 cells at 48 h after infection as previously described¹.

The binding of mRNA to ribosomes was carried out with either ³H-labelled late Ad2 or SV40 mRNA in four successive incubation steps according to Levin *et al.*¹⁵ (Figs 3 and 4). Late Ad2 mRNA prepared from KB cell polyribosomes forms the 80S initiation complex with T22 and KB cell ribosomal wash (Fig. 3a and b) but not with GC7 and C14 cell ribosomal wash (Fig. 3c and d); GC7 cells are also a line of AGMK cells, non-permissive to adenoviruses. A small shoulder observed at 80S in Fig. 3c and d may indicate the presence of a small amount of cellular mRNA in Ad2 mRNA preparation. The ribosomal wash prepared from a permissive monkey kidney cell line, Vero cells, formed a smaller amount of the 80S complex (Fig. 3e), which may reflect the lower susceptibility of Vero cells to adenovirus infection than KB and T22 cells. Late Ad2 mRNA prepared from the cytoplasm of infected C14 cells formed the complex with the T22 cell ribosomal wash with the same efficiency as late Ad2 mRNA prepared from polyribosomes of the infected KB cells (Fig. 3f) indicating the functional integrity in formation of the 80S complex of late

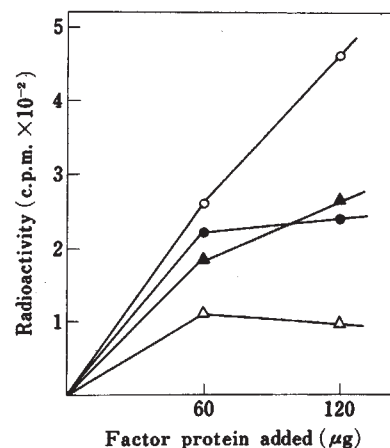


Fig. 5 Formation of methionyl-puromycin. The reaction mixture (0.3 ml) for formation of the 80S initiation complex containing ³H-met-tRNA (about 50 pmol, 20,000 c.p.m.) and either 8 µg of late Ad2 mRNA or 20 µg of poly(AUG) (Sigma) was incubated at 37°C with increasing amounts of ribosomal wash proteins as described in Fig. 3. Puromycin was then added to a final concentration of 1 mM (final volume 0.5 ml) and incubation was continued at 37°C for 30 min. The reaction was terminated by the addition of 0.5 ml of 0.2 M sodium phosphate buffer (pH 8.1) and ³H methionyl-puromycin was extracted²³ with 1.5 ml of ethyl acetate saturated with the same buffer. An aliquot (1 ml) of the ethyl acetate layer was counted in 10 ml of Bray's scintillator. ○, Ad2 mRNA, T22 cell ribosomal wash; △, Ad2 mRNA, C14 cell ribosomal wash; ●, poly(AUG), T22 cell ribosomal wash; ▲, poly(AUG), C14 cell ribosomal wash.

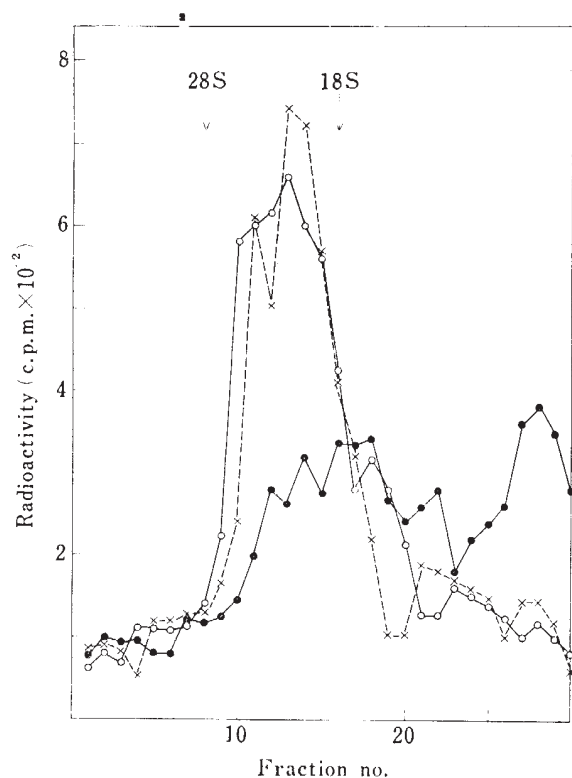


Fig. 6 Size distribution of late Ad2 mRNA after binding reaction. The reaction for formation of the 80S initiation complex with ^3H -labelled late Ad2 mRNA was carried out as described in Fig. 3 with ribosomal wash proteins from either T22 cells (○) or C14 cells (●). The reaction was terminated by the addition of 0.5% SDS and the mixture was centrifuged in a 15–30% (w/w) SDS–sucrose gradient at 24,000 r.p.m. for 18 h at 28°C in a Spinco SW 25.1 rotor. Each fraction was counted after TCA precipitation. ×, ^3H -labelled late Ad2 mRNA was centrifuged without reaction.

Ad2 mRNA transcribed in non-permissive monkey cells. The formation of the complex depended on Met-tRNA_i. The inability of C14 and GC7 cell ribosomal wash to bind late Ad2 mRNA to ribosomes does not result from an inactivation of factor proteins. The 80S initiation complex with SV40 mRNA was formed with ribosomal washes from C14 and GC7 cells which are permissive to SV40 (Fig. 4). The ribosomal washes from T22 and C14 cells facilitated binding of poly(U) to ribosomes at low concentrations of Mg^{2+} with the same efficiency, indicating that both ribosomal washes contain initiation factors M1 and M2. The mRNA–protein complex slightly larger than 40S was formed with the ribosomal wash from both permissive and non-permissive cells (Figs 3 and 4). It is not yet clear whether this complex is the 40S initiation complex or the ribonucleoprotein particle formed between late Ad2 mRNA and proteins in the ribosomal wash.

Functional integrity of 80S complex

To demonstrate that the 80S complex formed with late Ad2 mRNA is active in the initiation of protein synthesis, the formation of methionyl-puromycin (met-puro) in the reaction mixture with the ribosomal wash from both T22 cells or C14 cells was examined. Formation of met-puro increased linearly with increasing amounts of T22 cell ribosomal wash (Fig. 5), whereas with C14 cell ribosomal wash it reached a plateau after a small increase. This difference was not observed when late Ad2 mRNA was replaced with poly(AUG). Addition of 120 μg each of C14 and T22 cell ribosomal wash to the binding mixture did not alter the amount of met-puro formed by T22 cell

ribosomal wash alone, indicating that an inhibitor of the formation of either the initiation complex or dipeptide is not present in C14 cell ribosomal wash. The stability of late Ad2 mRNA during the binding reaction was studied by examining the alteration in its size. The size distribution of late Ad2 mRNA in an SDS–sucrose gradient after the binding reaction with T22 cell ribosomal wash was the same as that of non-incubated late Ad2 mRNA (Fig. 6), whereas the binding reaction with C14 cell ribosomal wash resulted in the degradation of late Ad2 mRNA. RNase activity in the ribosomal wash was low and not significantly different for C14 and T22 cells. These results indicate that formation of the functional complex with ribosomes protects mRNA from degradation and that the factor present in the ribosomal wash of T22 cells is specific for late Ad2 mRNA.

Genesis and biological significance

These results provide the first evidence that the permissiveness and non-permissiveness of the cells to virus infection is regulated at the initiation of protein synthesis. They also offer a good system for characterising the nature of the initiation factor M3. Appearance of a new initiation factor responsible for late Ad2 mRNA translation in SV40-transformed simian cells may be the result of either modification of a pre-existing M3 factor or induction of a new M3 factor by the SV40 gene function. The SV40 helper function for growth of human adenoviruses in monkey cells is one of the virus early functions, since an early mutant of SV40, tsA7 (ref. 16), defective in virus DNA replication, expresses this function at the non-permissive temperature. An adeno 2-SV40 hybrid virus, Ad2+ND₁ (refs 17 and 18) in which only 18% of the SV40 DNA is integrated into Ad2 DNA, expresses the SV40 function to induce U antigen and to support adenovirus growth in simian cells. The factor itself may not be coded by the SV40 genome, however, as the early region estimated by hybridisation competition is too small^{19,20} to code various proteins and the SV40 induced-U antigen is localised in the nuclei of the infected cells. The factor may be synthesised as a result of derepression of the host cell genome by some unknown SV40 early gene product. Our findings indicate that the efficiency of the helper function in supporting synthesis of Ad2 virion antigen in simian cells previously infected with SV40 (24 h before infection of adenoviruses) depends on the multiplicity of SV40 infection and correlates with the efficiency of induction of cellular mRNA synthesis. The alteration of ribosomes in the SV40-infected simian cells so as to respond to late Ad2 mRNA to synthesise hexon is also multiplicity-dependent. These results support the assumption that the derepression of the cellular function is involved in the helper function. Derepression of the cellular function by SV40 may cause the pleiotropic changes in the cells which would result in the alteration of various cellular systems, such as the cell surface structure²¹ and the protein-synthesising system, causing the cell to become malignant.

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Echinomycin: a bifunctional intercalating antibiotic

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The interaction between echinomycin and circular DNA or sonicated rod-like DNA fragments shows that this antibiotic binds by a bifunctional mode of intercalation. Binding parameters for a variety of natural and synthetic DNAs vary widely, indicating that echinomycin interacts selectively with specific nucleotide sequences.

THE significance of twofold symmetry in the structures of small molecules which bind to DNA, itself characterised by a dyad axis of symmetry perpendicular to the helix axis, was emphasised by Sobell *et al.*¹ in their model for actinomycin-DNA binding derived from a crystallographic study of a 1:2 actinomycin-deoxyguanosine complex. Subsequent work has shown the importance of symmetry-related elements in the recognition of DNA sequences by repressors^{2,3}, restriction enzymes⁴ and possibly transcriptional factors^{4,5}, suggesting that principles of symmetry may prove to be a general feature of recognition processes involving DNA.

We were struck by the apparently perfect symmetry in the structure of the antibiotic echinomycin⁶ proposed by Keller-Schierlein *et al.*⁷. Echinomycin has long been known to bind to DNA^{8,9} but the nature of its interaction has remained obscure. It is an extremely potent inhibitor of RNA synthesis^{8–10}, some

four to five times more potent than actinomycin D *in vivo*¹⁰, and there is every indication that its antitumour and other biological activities result from its binding to DNA¹¹. During the course of our work, proton magnetic resonance studies^{3,2} suggested that echinomycin contained four more hydrogen atoms than expected from the published structure⁷. While the revised structure (Fig. 1) retains a twofold axis of rotational symmetry for the cyclic octapeptide dilactone and its attached quinoxaline-2-carboxylic acid chromophores, the sulphur-containing cross-bridge (replacing the dithian ring originally proposed⁷) introduces an element of asymmetry. In respect of this pseudo-symmetrical appearance, as well as the sequence of amino acid residues in the two halves, there are obvious parallels with the structure of actinomycin¹.

Specificity for double-helical DNA

Echinomycin is much less soluble in purely aqueous systems than actinomycin, which necessitated the development of a novel solvent-partition method to investigate its binding to nucleic acids. Representative results, full details of which will be published elsewhere, are shown in Fig. 2. These curves reveal that the binding is completely specific for DNA, not RNA, that single-stranded or heat-denatured DNAs bind less antibiotic than intact double-helical DNA, and that complete disruption

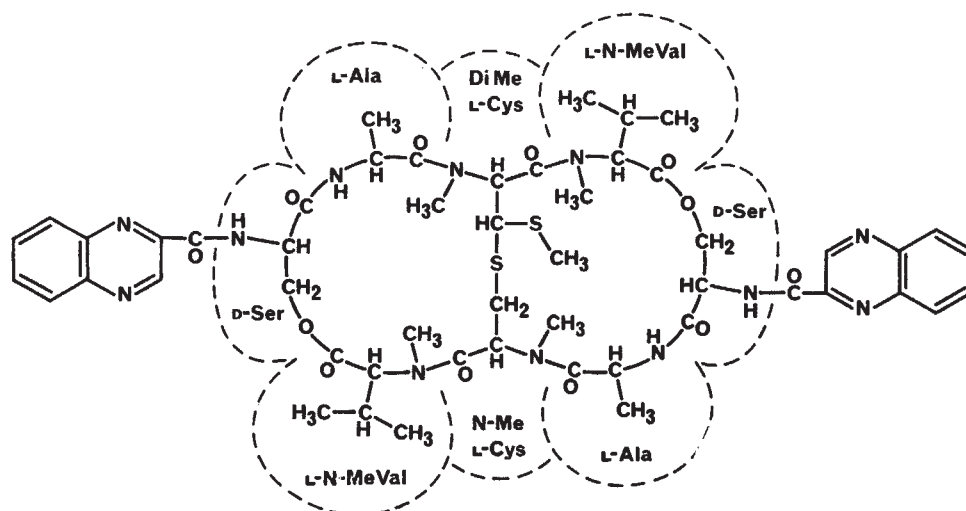


Fig. 1 Structure of echinomycin. Abbreviations: MeVal, methylvaline; Ser, serine; Ala, alanine; Cys, cysteine; DiMe-L-Cys, N,S-dimethyl-L-cysteine. The formula is a revision of that published previously⁷, based on new results from mass spectrometry and nuclear magnetic resonance spectroscopy^{3,2}.

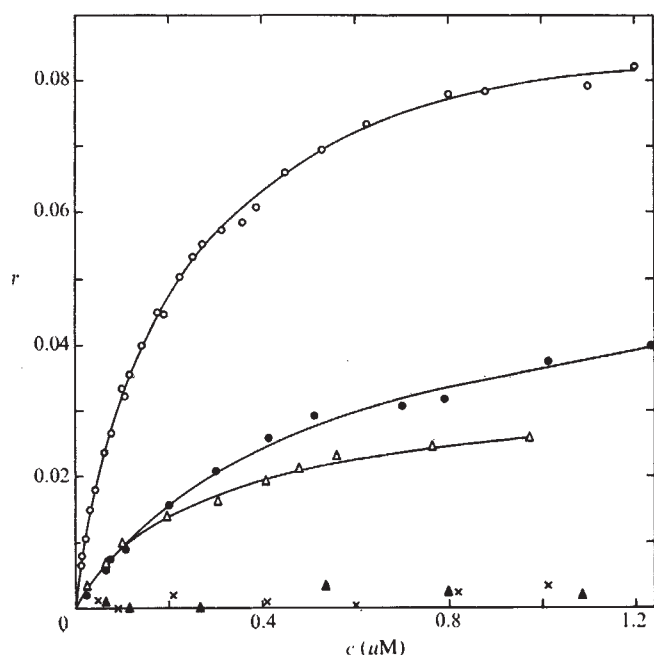


Fig. 2 Interaction between echinomycin and nucleic acids. The buffer (SHE) contained 2 mM HEPES, 10 μ M EDTA and 9.4 mM NaCl (pH 7.0 at 20°C; total ionic strength 0.01). Portions (3 ml) of nucleic acid solutions (182 μ M with respect to nucleotides) were shaken together with 4 ml of echinomycin dissolved in *iso*-amyl acetate for 2 h at 20.0°C to establish equilibrium. The phases were then separated by centrifugation at 2,000 r.p.m. for 30 min in an MSE Super Minor centrifuge. The free antibiotic concentration in the aqueous phase (c) was determined from the absorbance of the organic phase at 315 nm measured in a 40 mm light path semimicro cuvette, based on a molar extinction coefficient of 1.15×10^4 for echinomycin in *iso*-amyl acetate and its partition coefficient between this solvent and SHE buffer ($8.93 \pm 0.32 \times 10^{-3}$ at 20°C). The total antibiotic concentration in the aqueous phase was determined by measuring the absorbance at 325 nm after dissociating the complex by addition of an equal volume of dimethyl sulphoxide (DMSO). In 50% (v/v) DMSO–buffer mixtures the molar extinction coefficient was determined to be 1.24×10^4 at 325 nm irrespective of the nucleic acid concentration. The concentration of bound echinomycin was obtained by difference and expressed in terms of r , mol antibiotic bound per mol of nucleotides. $\circ$, Native calf thymus DNA sheared to produce fragments of approximately 18S free from single-stranded ends by the method of Pyritz *et al.*³⁰; $\bullet$, single-stranded circular DNA from bacteriophage fd; Δ , calf thymus DNA denatured by heating at 100°C for 10 min in SHE buffer followed by rapid cooling; $\blacktriangle$, calf thymus DNA denatured in the presence of 1% formaldehyde; $\times$, total ribosomal RNA from *E. coli* B.

of secondary structure by denaturation of DNA in the presence of formaldehyde abolishes the binding altogether. A Scatchard plot of the data for calf thymus DNA yielded a straight line up to an r value (antibiotic molecules bound per nucleotide) of about 0.06; the slope and intercept of this line gave an apparent association constant K_{ap} of $6.0 \pm 0.3 \times 10^6$ M⁻¹ and the number of sites per nucleotide (n) as 0.087, equivalent to one binding site per six base pairs in this DNA. Values of K_{ap} and n for the heat-denatured DNA were $4.0 \pm 0.4 \times 10^6$ M⁻¹ and 0.032, respectively; for fd DNA they were $2.2 \pm 0.2 \times 10^6$ M⁻¹ and 0.053. These parameters, two to three times lower than those for the native double-helical DNA, undoubtedly reflect the limited interaction between echinomycin and 'hairpin' helical segments present in the single-stranded DNAs; witness the lack of interaction after formaldehyde treatment. These results corroborate the qualitative conclusions of earlier workers^{8,9} and compare well with parameters reported for the binding of actinomycin to DNA^{12,13}.

Bifunctional intercalation

The quinoxaline chromophores of echinomycin are similar in size to the quinoline chromophore of chloroquine, which is

known to bind to DNA by intercalation^{14,15}. Their symmetrical disposition with respect to the octapeptide ring, together with the apparent structural and functional resemblance between echinomycin and actinomycin, suggested that both chromophores might be capable of intercalation, in a symmetry-related fashion, at the same time. A double-intercalation event would be expected to result in twice the unwinding of the helix caused by binding of a monofunctional intercalating agent¹⁵, twice the extension along the helix axis¹⁶, and markedly enhanced binding to negatively superhelical circular DNA at low values of r (ref. 17). These predictions have been verified.

Figure 3 compares the unwinding of the helix associated with binding of echinomycin to that caused by ethidium, an established (monofunctional) intercalating drug^{15,18,19}. Echinomycin removes and reverses the supercoiling of circular PM2 DNA in decisive fashion, with an equivalence binding ratio¹⁵ of 0.028 ± 0.004 antibiotic molecules bound per nucleotide. Under the same conditions equivalence occurs with 0.051 ± 0.007 ethidium molecules bound per nucleotide. Thus the helix-unwinding angle of echinomycin is 1.82 ± 0.30 times that of ethidium, or 21.9° per bound antibiotic molecule if the estimate of 12° unwinding for ethidium¹⁸ is accepted^{15,19}. This is the first time a drug has been found which unwinds the DNA helix by an angle larger than does ethidium, the actual value being almost double.

Figure 4a shows the extension of the helix caused by binding of echinomycin, 1.87 ± 0.05 times the theoretical extension of 3.35 Å required to accommodate a single aromatic ring system, corresponding to a lengthening of about 6.3 Å per bound antibiotic molecule. Comparable experiments with proflavine and ethidium were reported to yield a value of 2.7 ± 0.2 Å (refs 16 and 20). Again the value for echinomycin is twice as high.

Figure 4b compares binding isotherms for the interaction between echinomycin and closed or nicked circular PM2 DNA. They cross at an r value of about 0.027, confirming the equivalence binding ratio determined in the sedimentation experiments (Fig. 3a) and corresponding to an unwinding angle 1.89 times

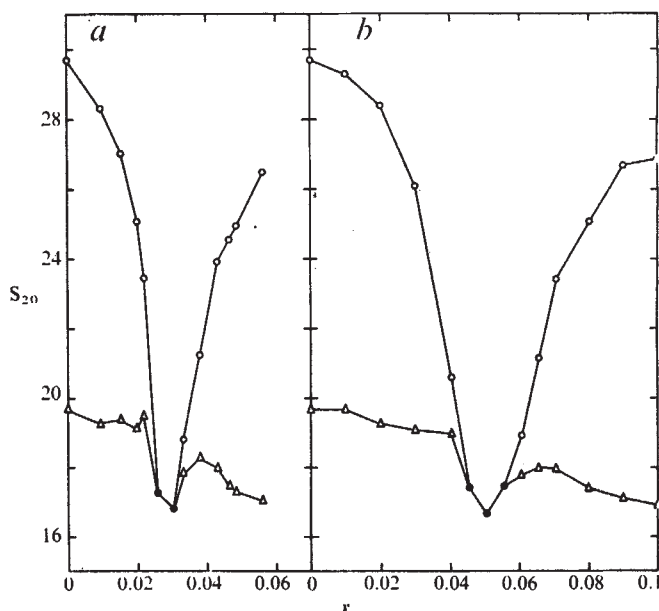


Fig. 3 Effects of (a) echinomycin and (b) ethidium bromide on the sedimentation coefficient of PM2 DNA in SHE buffer. The DNA preparations³¹ contained 80–90% closed circular duplex molecules. $\circ$, S_{20} of closed circles; Δ , that of nicked circles; $\bullet$, weight-average S_{20} when the relaxed closed circular molecules cosedimented with the nicked circles. Ultracentrifugation experiments and spectrophotometric measurements of ethidium–DNA binding were as previously described¹⁵. Echinomycin–DNA complexes were formed by adding small volumes of antibiotic dissolved in acetone to the DNA in SHE buffer; the acetone was then removed under reduced pressure. The binding ratio (r) was determined from a curve constructed as in the legend to Fig. 2. Sedimentation coefficients are uncorrected values determined directly at 20°C.

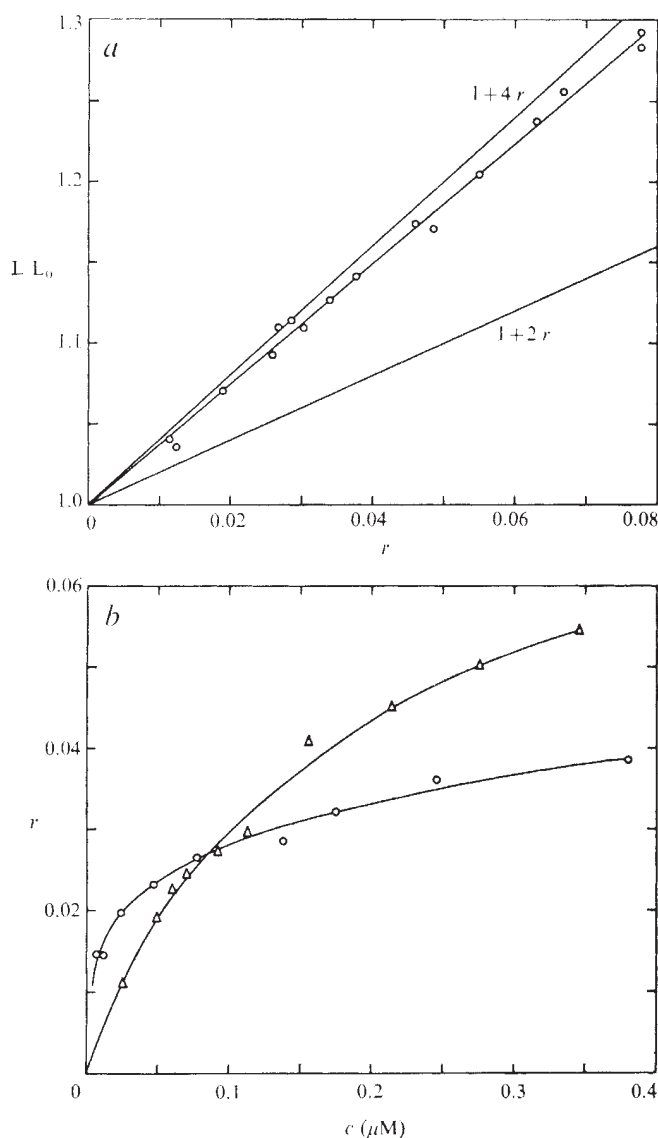


Fig. 4 *a*, Relative length increase L/L_0 of echinomycin-DNA complexes as a function of the binding ratio. L/L_0 was calculated from viscosity measurements on sonicated rod-like DNA fragments in SHE buffer at 20°C using a capillary viscometer as described by Cohen and Eisenberg¹⁶. The DNA fragments were prepared by sonicating calf thymus DNA to a molecular weight of 5.4×10^5 in an MSE 150 W ultrasonic disintegrator; their intrinsic viscosity in the absence of antibiotic was 3.9 dl g^{-1} . Complexes of known binding ratio (r) were prepared as in Fig. 2. The line fitted to the points is a least-squares line, constrained to pass through the origin, of slope $1 + 3.73 r$. Also shown are theoretical lines corresponding to the lengthening expected for ideal monofunctional intercalation ($1 + 2r$) and ideal bifunctional intercalation ($1 + 4r$). *b*, Binding of echinomycin to closed (○) and nicked (Δ) circular PM2 DNA in SHE buffer, determined as described in Fig. 2. The sample of nicked circles was generated by repeated freezing and thawing.

greater than that of ethidium. More important, the enhanced binding to closed circles at ratios below the crossover, and restricted binding above it, are clearly seen. The differential binding to the two forms of DNA at low r is considerably more marked than that reported for ethidium²¹, as expected if the ratio of equilibrium constants for closed circles compared to nicked circles (K_I/K_{II}) is an exponential function of the unwinding angle¹⁷. Equations (4) and (7) of Davidson¹⁷ predict a value of $K_I/K_{II} = 5.8$ for echinomycin binding to a typical closed circular DNA at $r \rightarrow 0$, based on an unwinding angle of 22°. One consequence of this is that any biological property resulting solely from preferential interaction with negatively superhelical

circular DNA would be amplified some 2.23 times with echinomycin ($K_I/K_{II} = 5.8$) in comparison with ethidium ($K_I/K_{II} = 2.6$)¹⁷. Furthermore, detailed measurements of the interaction between echinomycin and a range of natural and artificially-closed circular DNAs should enable more accurate estimation of such parameters as the superhelix free energy²¹, in addition to providing a more sensitive probe for the biological relevance of circularity in DNA.

Nature of the binding site

The unwinding angle and extension of the helix per bound echinomycin molecule, being double the values observed with classical intercalating drugs, lend strong support to the argument that each chromophore participates in an intercalation process symmetrically related to the binding site of its partner. The two intercalation sites are presumably distinct, because it is practically inconceivable that both chromophores might be accommodated in the potential space between one set of adjacent base-pairs. The symmetry argument would therefore predict that whatever constraints apply to the intercalation site for one chromophore should apply in antiparallel fashion to the site for the other. In the extreme case where each chromophore demanded one specific site out of the ten distinguishable types of potential space¹⁹, the specificity of binding of the antibiotic as a whole would be phenomenal: one in 40 or one in 100 possible locations along the length of a random sequence would be acceptable, depending on the number of base pairs (1 or > 1) between the intercalated chromophores.

Echinomycin lacks that extreme specificity. Nevertheless, there is some form of subtle specificity in its interaction with DNA. Binding parameters determined for a range of natural DNAs having G+C contents from 29 to 72% yielded values of K_{ap} from $4.5 \times 10^6 \text{ M}^{-1}$ to $4.0 \times 10^7 \text{ M}^{-1}$ with a tendency for the DNAs richer in G+C to bind the antibiotic more tightly. However, two DNAs of identical base composition (*Escherichia*

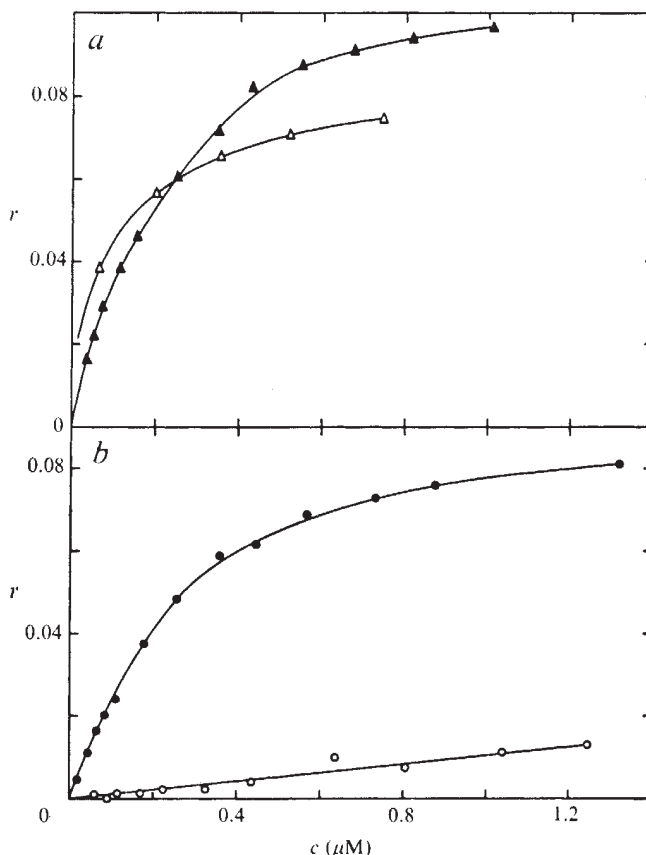


Fig. 5 Interaction between echinomycin and poly(dA)·poly(dT) (○), poly(dA-T); (●), poly(dG)·poly(dC); (Δ) and poly(dG-C); (▲) in SHE buffer at 20°C, measured as in Fig. 2.

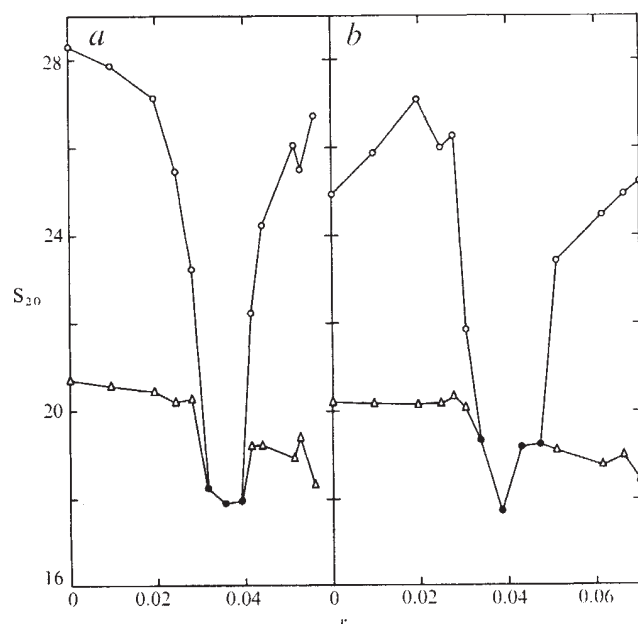


Fig. 6 Effect of echinomycin on the sedimentation coefficient of PM2 DNA at ionic strength 0.1 (a) and 0.5 (b). Symbols and methods as described in the legend to Fig. 3. The buffers were SHE supplemented with NaCl to the required ionic strength. At ionic strength 0.1 equivalence occurs with 0.036 ± 0.006 echinomycin molecules bound per nucleotide; at ionic strength 0.5 the value rises to 0.041 ± 0.007 . In the same buffers equivalence binding ratios for ethidium are 0.055 ± 0.005 and 0.048 ± 0.009 , respectively²⁸.

coli and *Salmonella typhimurium*; both 50% G+C) yielded significantly different association constants. Numbers of sites derived from Scatchard plots fell within the range 0.054–0.087 sites per nucleotide, but no correlation with the base composition was apparent.

Striking differences were found when the binding to synthetic DNA polymers was investigated (Fig. 5). Three polymers bound the antibiotic well, albeit with widely different binding constants that is, poly(dG)·poly(dC), poly d(G-C) and poly d(A-T). By contrast, poly(dA)·poly(dT) showed barely detectable binding (Fig. 5); neither did poly(dI)·poly(dC), poly d(I-C) and poly(rA)·poly(rU), notwithstanding their regular helical secondary structure. The data for the three polymers which showed strong interaction were re-plotted according to the statistical mechanical treatment of Zasedatelev *et al.*²², from which the number of base pairs occluded per bound echinomycin molecule was calculated to be three for poly d(A-T) and poly d(G-C) but nearer four for poly(dG)·poly(dC). The interactions with synthetic polydeoxyribonucleotides will be discussed fully elsewhere, but it should be noted that the capacity of a given polymer to bind echinomycin is not simply correlated with its precise helical form²³, nor with the extent to which its helical structure resembles the B-form of natural DNAs. Since coliphage T2 DNA, whose major groove is largely filled by glucosyl substituents, bound the antibiotic well ($K_{ap} = 1.5 \pm 0.1 \times 10^7 \text{ M}^{-1}$; $n = 0.054$) it is likely that the binding site for the non-intercalated peptide portion of echinomycin lies in the minor groove, as is believed to be true for actinomycin^{1,13}. If so, the presence and/or orientation of the 2-amino group of guanine is likely to have important influence on the binding reaction, as is known to be the case with actinomycin^{1,13,24}. This is clearly implied by the different binding properties of polymers apparent in Fig. 5.

Physical characteristics of the binding reaction

The equilibrium constant for binding to native calf thymus DNA at ionic strength 0.01 showed no detectable variation

with temperature between 20 and 40°C. Thus ΔH must be close to zero and the binding reaction is driven by a large positive entropy change, $+31 \text{ e.u.}$ per mol of echinomycin bound. These parameters are in exact agreement with those reported for actinomycin binding under almost identical conditions¹². Unlike actinomycin, however, the solubility of echinomycin in aqueous systems is practically independent of ionic strength and temperature and remains about $5 \mu\text{M}$. This limited solubility corresponds to a negative entropy change of approximately 25 e.u., suggesting that the major driving force for the binding reaction is the transfer of echinomycin from an aqueous phase into a hydrophobic environment.

When the salt concentration is raised the character of the binding reaction changes. At ionic strength 0.1 the value of ΔS falls drastically to near zero and the ΔH of binding becomes $-8.5 \pm 2.6 \text{ kcalorie mol}^{-1}$. At the same time the equivalence binding ratio for PM2 DNA increases relative to that of ethidium, yielding an apparent unwinding angle of $18.4 \pm 3.0^\circ$, 1.54 ± 0.25 times that of ethidium (Fig. 6a). In the same solvent, viscometric measurements like those presented in Fig. 4a reveal an extension of the helix corresponding to $1 \pm 3.0 r$, that is, 1.50 ± 0.05 times the theoretical extension for a single intercalation event. On raising the ionic strength to 0.5 the trend is continued (Fig. 6b). The unwinding angle becomes 1.18 ± 0.19 times that of ethidium, and the helix extension falls to 1.16 ± 0.03 times the value for ideal monofunctional intercalation. The agreement between these totally independent assays of the effect of echinomycin binding on the DNA helix leaves little room for doubt that at ionic strengths 0.01, 0.1 and 0.5 the binding occurs by bifunctional, sesquifunctional, and almost monofunctional intercalation respectively.

Structural requirements

The cross-bridged symmetrical octapeptide ring of echinomycin is not merely a convenient handle on which two potentially intercalative chromophores are mounted. In order to investigate the possible role of the chromophores in isolation we studied the interaction of quinoxaline-2-carboxamide (a gift from Dr A. Dell) with DNA. This compound (Q2C) produced no detectable effect on the sedimentation of closed circular PM2 DNA at Q2C/nucleotide ratios up to 1:1 in buffer of ionic strength 0.01. Moreover, binding was undetectable by perturbation of ultraviolet absorption spectra or equilibrium dialysis under conditions²⁵ where interaction characterised by a binding constant as low as $2 \times 10^3 \text{ M}^{-1}$ would have been measurable, a factor of 3,000 lower than the binding constant for echinomycin itself. Clearly the tight binding of echinomycin to DNA cannot derive solely from intercalation of the chromophores but must involve interactions with functional substituents elsewhere in the intact molecule, that is, on the peptide ring. Nevertheless, although the chromophores alone do not appear to be able to bind to DNA they undoubtedly play a vital part in dictating the geometry of the antibiotic-DNA complex on which such sequence-specificity as exists must depend. Their intercalation causes the helix to unwind and it is in this distorted configuration that interactions between the peptide portion of the antibiotic and substituents in the DNA must be optimised. Such interactions with the undistorted helix may be unfavourable or indeed impossible.

The role of the bridge across the two antiparallel halves of the peptide ring may be more critical. Echinomycin belongs to a family of chemically related metabolites produced by streptomycetes, known generically as quinoxaline antibiotics^{11,26}. This family includes the triostins, which are also antibacterial and antitumour agents¹¹ capable of powerfully inhibiting RNA synthesis¹⁰. Triostin A is structurally homologous to echinomycin except that the cross-bridge is formed by N,N'-dimethylcystine²⁶. Triostin C differs further in that the N-methyl-valine residues are replaced by N, γ -dimethyl-L-alloisoleucine²⁶. Both triostins A and C were found to remove and reverse the supercoiling of PM2 DNA but the ratios required to reach equivalence, while still lower than the equivalence ratio for

ethidium, were higher than the equivalence ratio for echinomycin.

Implications

Although drugs which bind to DNA are of paramount importance in several areas of chemotherapy²⁷, not least the treatment of cancer, their capacity to discriminate between DNAs from different sources is minimal if not actually non-existent¹⁹. Few exhibit anything approaching sequence-specificity, the preference of actinomycin for binding to GpC sequences being a possible exception^{1,24}. Echinomycin appears to be little better, but at least its capacity for bifunctional reaction heralds the possibility of gaining more genuine sequence specificity. Absolute specificity might be achieved through direct interaction between functional groupings on the base-pairs and the drug molecule, or indirectly via recognition of a local conformational peculiarity of the DNA associated with a particular nucleotide sequence^{23,28}. There is evidence that either or both of these phenomena are features of echinomycin-DNA interaction, and we are currently exploring the details in order to develop a precise molecular model for the binding process. Once the exact intermolecular contacts are established, and the importance of the symmetrical structure of the antibiotic is clarified, the way will be open to devise means of enhancing the selectivity of binding. Naturally-occurring analogues with various amino acid substitutions exist, and some analogues with modified chromophores have been synthesised¹¹.

A detailed molecular model should also throw light on two outstanding problems concerning the intercalation reaction itself, that is, the origins of the neighbour-exclusion phenomenon (if it exists)^{19,22,29} and the absolute magnitude of helix-unwinding angles which are currently referred to an assumed value of 12° for ethidium^{15,18,21,25}. It may also add to our understanding of how macromolecules such as repressors, transcriptional factors, restriction enzymes and polymerases recognise specific loci in DNA, and the role of symmetry relationships in such recognition.

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New observations of the angular diameter-redshift relation for radio sources

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Measurements of the angular sizes of radio source components in the range 0.1"-1.0", using the technique of interplanetary scintillation, reveal an absence of small-diameter sources at the largest redshifts. The cosmological implications of this result are discussed.

It is well known that the angle subtended by a 'standard rigid rod' varies with redshift in a manner which depends upon the assumed cosmological model. In classical world

models this angle exhibits a minimum near $z=1$ which contrasts with the linear decrease of angle obtained for Euclidean geometry. Attempts to use the observed angular diameters of optical galaxies and radio sources to discriminate between different cosmologies have run into various difficulties. The subtleties of applying the test to galaxies have been described by Sandage¹ and although progress has been made towards solving some of the problems² the observations do not yet extend to large enough redshifts for a decisive result to be obtained. Radio

sources are characterised by a large spread of physical sizes which has made them unsatisfactory as rigid rods. When the largest angular sizes of radio sources having steep spectra are plotted against redshift an approximately linear decrease is found to a redshift $z \sim 2$. Miley³ has suggested that this apparent Euclidean behaviour might be due to cosmological evolution such that the physical size of a source is smaller at earlier epochs.

Here we use new observations made at a higher angular resolution by the method of interplanetary scintillation.

Scintillating sources as standard rods

A radio source will not scintillate strongly unless it contains components which, individually, subtend an angle of less than $2''$ in any direction and our study is therefore confined to compact features within radio sources, in contrast to their overall angular size.

The observations are taken from a recent survey of scintillating radio sources at 81.5 MHz which has been fully described elsewhere⁴, and we also make use of some additional measurements at 151 MHz. Amongst the 1,500 sources listed in this survey is a complete sample of ~ 200 3CR sources, a substantial fraction of which have been optically identified and for which the redshifts are therefore known.

By restricting our attention to strongly scintillating sources (those which emit at least 40% of their 81.5 MHz flux from components having an angular size $< 2''$) which are also non-variable, we suggest that we obtain a more closely defined range of objects at large redshifts than has been available hitherto for use in the angular diameter *versus* redshift test. Our reasons are as follows:

(1) An investigation of the nature of this sample has shown that strong scintillators are sources of the highest intrinsic luminosity⁵.

(2) It may be deduced from the spectra of the sources, and by combining scintillation results with high resolution maps made with the Cambridge 5-km telescope, that for sources with steep spectra ($\alpha > 0.75$) the compact features of size $< 1''$ are nearly always located in the outer components of typical 'double' radio galaxies. In addition, the ratio (component size/component separation) can be inferred for sources whose components are unresolved by mapping, and it is notable that the values inferred are in fair agreement with those obtained directly from mapping the larger sources⁶. This shows that the source morphology is preserved over a wide range of angular size. The physical sizes of the compact features are typically 2–5 kpc and they are probably similar to the bright "heads" which have been found in Cygnus A (ref. 7); in strong scintillators, however, these regions must emit a substantial fraction of the total flux.

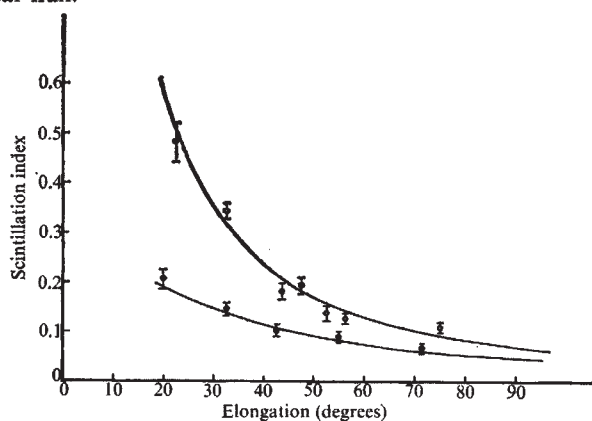


Fig. 1 The observed variation of scintillating flux density ΔS at 151.5 MHz as a function of solar elongation ϵ , for two typical sources. Also shown are theoretical curves for Gaussian sources of given angular size. ○, 3C446 (0.1''); ●, 3C275 (0.7').

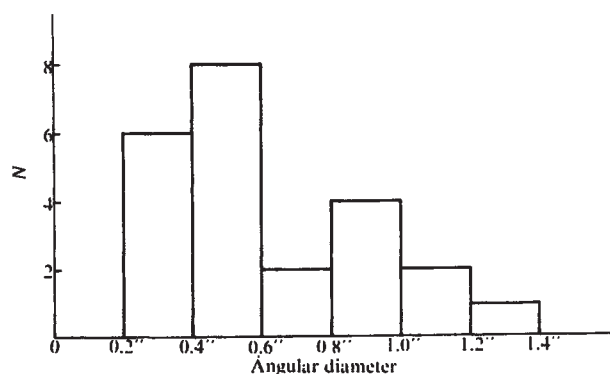


Fig. 2 The distribution of angular diameters for 23 strongly scintillating sources at 81.5 MHz.

(3) In a small number of cases strong scintillators are associated with objects having an overall angular size of less than $1''$, such as 3C273, 3C279 and 3C446. Sources of this type often exhibit temporal variability at centimetre wavelengths, and have flat radio spectra. These highly compact sources may therefore usually be distinguished, in the absence of angular size data, from the typical double radio sources which have steeper spectra and which are non-variable. Such sources have been excluded from our sample.

The observations

Measurements both of angular diameter, and of the fraction of the total flux in components of size $< 2''$, can be made with fair accuracy by the method of interplanetary scintillation on sources of high flux density, provided that they can be observed over a wide range of solar elongation ϵ . The method is illustrated in Fig. 1 which gives two examples of how the r.m.s. scintillating flux density ΔS varies with ϵ . The angular diameter is obtained by comparing the observations with theoretical curves based on a model of the interplanetary plasma⁸. The value is not sensitive to the assumed source model⁹ and the results that we use are based on a circularly symmetrical Gaussian model⁴. If two or more scintillating components of equal size, spaced by at least $1''$, are present in a given source (as might be the case for a typical double radio source) the derived angular size is unchanged but the fraction of the total flux in these components is slightly underestimated. The overall reliability of the scintillation technique has been checked by comparison with VLBI methods¹⁰ at the high resolution limit of $0.1''$ – $0.2''$ and by comparison with maps obtained with the 5-km telescope at the low resolution limit of $1''$ – $2''$. We find no reason to doubt the validity of results such as those given in Fig. 1.

In the sample of 200 3CR sources there are (classified as A sources in Readhead and Hewish's catalogue⁴) 23 strongly scintillating sources for which curves of a similar quality to those shown in Fig. 1 have been obtained. This sample excludes sources lying within $\pm 20^\circ$ of the galactic plane where interstellar scattering can give rise to a significant increase in the angular size at 81.5 MHz (ref. 11). The distribution of angular diameters derived from the scintillation curves is shown in Fig. 2.

The rapid decrease in the number of sources having sizes $> 1''$ is to be expected because the degree of scintillation falls steeply in this range. A more surprising feature is the peak in the distribution at about $0.4''$. This value is twice the resolution limit of $0.2''$ and is unexpected since, *a priori*, there is no reason to suppose that there will not be a very wide dispersion in the angular sizes of the compact components. If the actual sizes were more or less uniformly scattered over the range below $1.0''$ then we should expect the numbers to increase rapidly towards the resolution limit because the use of the scintillation technique

introduces a strong selection effect favouring the detection of sources having the smallest angular size.

What other observational selection effects might account for the observed distribution? One possibility might be that the compact components have roughly the same physical size and luminosity, and that they subtend an angle of $0.3''$ – $0.4''$ when they lie at a distance such that their scintillating flux density falls near the limit for classification as an 'A' source in the catalogue. In that case, however, there should be a correlation between flux density and angular size which is certainly not observed. Thus this possibility cannot explain the observations.

A more important possibility is that we are measuring an apparent size due to angular scattering in the interstellar medium¹¹. Our recent measurements (to be published shortly) of this effect show that an extragalactic point source would have an apparent angular diameter of $0.17'' \pm 0.3'' (\sin b^{11})^{-1/2}$, which gives a mean value of $0.2''$ for a random distribution of sources at latitudes $b^{11} > 20^\circ$. A further check was made by observing a number of sources in the sample at 151 MHz, where the interstellar scattering would be nearly four times smaller. At 151 MHz the angular diameters were typically only 20% smaller, as shown in Fig. 3, in good agreement with the above formula for source broadening at 81.5 MHz. It therefore follows that the values at 81.5 MHz are only slightly modified by interstellar scattering and this is not sufficient to produce a maximum in the distribution near $0.4''$. We conclude that the distribution of angular diameters is real and cannot be explained by observational selection or other spurious effects.

The angular diameter—redshift relation

The angular diameters of the scintillating components are shown as a function of redshift z in Fig. 3, where the limits of the scintillation technique are also indicated. When a given source was observed at 81.5 MHz and 151 MHz both values are shown, and the differences can be ascribed to interstellar scattering as mentioned earlier. It is not surprising, in view of the limitations of the technique, that the measurements show little obvious redshift dependence although there is a tendency for the mean diameter to increase at large z . There is, however, a remarkable deficit of sources having diameters less than about $0.3''$ in the range $0.7 < z < 1.6$ (denoted by the quadrilateral in Fig. 3).

The sloping lines in Fig. 3 show the expected trend, on the basis of Euclidean geometry, for a population of sources whose physical sizes are independent of redshift. If the population in the range $0.2 < z < 0.5$ is typical then we expect to find about six sources having diameters $< 0.2''$ in the range $0.7 < z < 1.6$. Instead of the expected number, clustered near the resolution limit of $0.2''$, no sources are observed, even though this is the region where the scintillation technique has the greatest detection sensitivity.

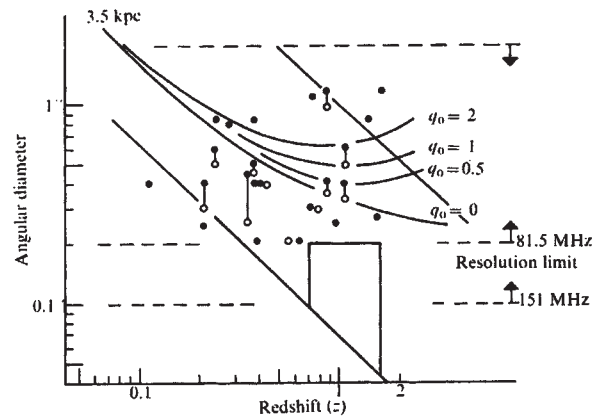


Fig. 3 The angular diameter versus redshift relation for strongly scintillating sources. ●, 81.5 MHz; ○, 151 MHz.

A straightforward explanation of these results can be given if a non-Euclidean world model is assumed. The curves in Fig. 3 show the angle subtended by a rigid rod of length 3.5 kpc for different cosmological models assuming $H = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$. The observations do not seem to fit the curve for a deceleration parameter $q_0 = 0$, but are consistent with $0.5 < q_0 < 2.0$. Any tendency for the linear sizes of scintillating components to vary with epoch will, of course, modify the derived value of q_0 . If q_0 is to be smaller, for example, the linear size must increase with z .

It is clearly desirable to obtain information on a larger sample of sources but it is already evident that the angular sizes of scintillating components in radio sources are hard to explain by Euclidean geometry. Strongly scintillating radio sources appear to provide the best rigid rods that are currently available for cosmological tests.

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letters to nature

X rays predicted from flares of UV Ceti stars

KNOWN UV Ceti flare stars are all located within 20 pc of the Sun, and their distribution seems to be isotropic. They are cool, low-luminosity objects with masses less than $0.5 M_\odot$. Both optical¹ and radio² radiations have been observed during flare events. X rays have also been predicted^{3,4}, but no positive

observations have yet been reported. We present here new estimates of X-ray emission, based on the ratio between X-ray and radio flux from solar bursts. It is anticipated that the intensities of the X-ray flares will be more than $10 \text{ photons cm}^{-2} \text{ s}^{-1}$; within the range of sensitivities of present satellite detectors (approximately 0.5 – $0.01 \text{ photon cm}^{-2} \text{ s}^{-1}$ for a flare duration of 3 min in the energy range 1–10 keV).

Table 1 Predictions and upper limits of X-ray emission during flares of UV Ceti stars

X-ray energy (keV)	Description	X-ray luminosity (erg s ⁻¹)	X-ray flux at Earth (photons cm ⁻² s ⁻¹)
> 4.1	Scaling model based on the ratio solar X-ray: solar radio (210 MHz)		
1.6–12.4	UV Ceti, 10 Jy (ref. 10)	7.2×10^{30}	1
0.62–1.6		1.5×10^{32}	40
		5×10^{32}	350
> 10	Scaling model based on the ratio solar X-ray: solar optical (3,500–6,500 Å)		
1.6–12.4	UV Ceti, $\Delta m_V = 1$ (ref. 4)	3×10^{24}	2×10^{-7}
0.62–1.6		2.4×10^{28}	10^{-2}
		1.8×10^{28}	10^{-2}
> 10	Model UV Ceti, $\Delta m_V = 1$ (ref. 3)	3.4×10^{31}	2.6
> 7.7	Measured 3 σ upper limit, OSO-3	3.6×10^{33}	300
	YZ CMi, $\Delta m_U = 1.5$ (ref. 15)		
	Upper limits from measurements of the Galactic component of the diffuse X-ray background and the assumed flare-star parameters for UV Ceti, $\Delta m_V = 1$		
7.7–12	(ref. 16)	7×10^{29}	0.05
2–10	(ref. 17)	10^{31}	3
0.2–2	(ref. 18)	6×10^{31}	150
1–10	Minimum 3 σ source detectability, MIT OSO-7; UV Ceti flare of 3 min duration	10^{30}	0.5

Variability in the types, intensities, and durations of stellar and solar flares makes uncertain both optical/X-ray and radio/X-ray scaling comparisons. Uncertainties in the optical scaling model of Kahler and Shulman⁴ arise, however, from two additional sources. First, the optical excess from solar flares is difficult to measure and has been reported for only a few large flares. Second, because of the limited range of optical band widths in which flare stars have been observed, direct comparisons are not possible and bolometric comparisons between stellar and solar flare emissions are dependent on the model used. Predicting X-ray emission using the ratio between solar X-ray radiation and solar radio radiation is less ambiguous because radio signals from solar flares and from flare star bursts have been observed at the same frequencies. Scaling UV Ceti-type emission by using that ratio is justifiable because of the similarities between solar and flare-star bursts and because of the temporal correlation between the X-ray emission and the radio burst signals observed from solar flares^{5,6}. Laboratory studies have shown the applicability of such scaling over remarkable ranges of the physical parameters⁷. Kahn⁸, using both plasma and synchrotron models, has shown that the observed characteristics of flares of UV Ceti stars are consistent with volumes, temperatures, and densities not much different from those suggested for the corresponding models of solar flares. Mullan⁹ has developed a model of the spots observed on red dwarf flare stars which indicates that the magnetic fields responsible for the star spots have strengths of approximately 20 kgauss. Such fields, approximately 10 times as strong as those observed in sunspots, would provide a factor of 100 in the energy available for release in flares over comparable volumes. The factor of 100 also helps to account, albeit only slightly, for the much greater radio luminosity of flare-star bursts relative to those of the Sun. Lovell² has pointed out that many flare-star radio bursts are 10^4 – 10^6 times more intense than the most intense solar radio flares.

Our predictions, based on the ratio between X-ray emission and radio emission from a particular solar flare¹⁰, are presented in Table 1 together with other predictions and observational upper limits. For comparable energy ranges, the X-ray to radio scaling results yield a factor of 10^4 more X-ray emission than the X-ray to optical scaling suggested by Kahler and Shulman⁴. The solar-flare data we have used does not quote a value for the flux above 10 keV; we have, however, estimated the X-ray emission for energies above 10 keV by scaling the predictions of Kahler and Shulman⁴ by the factor of 10^4 . We

obtained an X-ray luminosity of 3×10^{28} erg s⁻¹ and an X-ray flux of 2×10^{-3} photon cm⁻² s⁻¹ at Earth for a 10 Jy radio burst (210 MHz) of UV Ceti. Although more optimistic than the predictions of Kahler and Shulman⁴, these values lie a factor of 10^3 below the predictions of Grindlay³ and, in this energy range, outside the levels of detectability of present satellites.

Using the requirement that the integrated X-ray flux from all UV Ceti flare stars should not exceed the observed, diffuse X-ray background, and assuming the mean flare-star parameters cited by Edwards¹¹, we have calculated upper limits for the X-ray emission of a $\Delta m_V = 1$ flare of UV Ceti (Table 1). These values are 10^{-8} times the diffuse luminosity of the Galaxy in each energy range considered. The fluxes based on our scaling predictions do not exceed these limits by more than a factor of 10 in any of the energy ranges. Because of all the uncertainties in the flare-star parameters, especially in their Galactic distribution and density which have been determined only within 20 pc of the Sun, and because of the additional uncertainty in the frequency of occurrence of radio flare bursts, the disagreement is not significant. Depending on the details of their Galactic distribution, the UV Ceti flare stars may, in fact, satisfy the spatial, spectral, and flux characteristics required of unresolved sources of the observed, diffuse X-ray background¹².

A search for coincident optical, radio, and X-ray coverage of flare activity in UV Ceti stars is now under way. As indicated by the final entry in Table 1, the MIT OSO-7 X-ray detectors have sufficient sensitivity to refute or to confirm the more optimistic predictions if coincident coverage is obtained. Rates of radio flare bursts above a threshold of 0.2 Jy are comparable to the rates of optical flares above a threshold of approximately $\Delta m_U = 0.5$ for YZ CMi and AD Leo¹³. A coordinated optical and radio observing programme, using high time-resolution photometric techniques (see ref. 14) has been carried out; and we are examining the OSO-7 data for coincident X-ray observations. Improvement in X-ray sensitivity by a factor of 10 can be obtained using the new, pointed X-ray observatories such as the Netherlands Astronomical Satellite (ANS) or the MIT SAS-C observatory which is scheduled to be launched next June. Prospects for coordinated observing programmes are being investigated.

In the case of solar fast-drift radio bursts (Type III), low energy (~ 4 keV) X-ray emission is more strongly correlated with the decimetre radio flux than with the metre radio flux, although considerable scatter exists in both populations

(S. Kahler, personal communication and ref. 19). Still better correlation has been found between the higher energy (>20 keV) X-ray flux and the 3 GHz radio flux observed in impulsive solar-flare events²⁰.

Unfortunately, radio observations of UV Ceti flare stars have been almost entirely at radio frequencies about or below 400 MHz. We hope that higher frequency radio observations will be possible and will soon be available.

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Positional agreement between 3U1706+32 and the cluster of galaxies A2241

UNIDENTIFIED X-ray sources at high galactic latitudes have been the subject of much discussion (see ref. 1 and refs therein). We call attention here to the existence of a rich Abell cluster of galaxies in the error box of the Uhuru high galactic latitude source 3U1706+32. The X-ray error box is rather large (9.8 square degrees), and the apparent intensity of the source is $R_x = 4.1 \pm 0.6$ counts s^{-1} in Uhuru counts². The cluster, Abell 2241, is located at 16 h 57.8 min +32° 37' (1950)³; it belongs

to distance group 3 and has an estimated redshift, $z \approx 0.07$ (ref. 3). The cluster is of richness group 0 (ref. 3) and has been classified⁴ as an irregular (I) cluster. The positional agreement between this cluster and 3U1706+32 was not reported in the survey of positional correlations between 3U X-ray sources and Abell clusters of galaxies of distance groups, $D \leq 3$, (ref. 5). The cluster A2241 should, therefore, be added as a possible identification to the list⁵ of 21 clusters which have been tentatively identified with X-ray sources; no additional Abell cluster of distance group 3 or nearer lies within Uhuru² high galactic latitude error boxes.

Although A2241 is located within the error box of 3U1706+32 and is therefore a candidate for identification with the X-ray source, we stress that a much improved X-ray position is required before a certain identification can be made. The X-ray luminosity of the cluster, assuming it to be at a redshift of 0.07, is about 10^{45} erg s^{-1} (for $H_0 = 55$ km s^{-1} Mpc $^{-1}$). This luminosity is somewhat higher than the X-ray luminosity range ($\approx 10^{43}$ to $\approx 3 \times 10^{44}$ erg s^{-1} ; see ref. 6) obtained for the other irregular clusters previously identified with X-ray sources, but is comparable with the luminosities of the brightest known regular X-ray clusters.

As is true for most of the other X-ray clusters, radio emission has also been observed from A2241; a double radio source, with a strength of 0.9 Jy at 1,445 MHz, is located in the projected area of the cluster⁷. An elliptical galaxy is located at the radio centroid⁷. It will be very interesting to learn whether or not the X-ray source is centered on this galaxy.

We consider that A2241 is a possible optical candidate for the high galactic latitude X-ray source 3U1706+32. A better measurement of position is however, required. It is also important to measure the cluster redshift so that, if the identification is correct, the X-ray luminosity can be calculated more accurately.

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Radio structure of the M87 jet

THE central region of M87 has a complex radio structure. There are at least three components^{1,2}, with diameters $\approx 0''.0013$, $\approx 0''.01$ and $\approx 0''.3$, coincident with the optical nucleus of the galaxy and on either side there are two elongated components, $\approx 25''$ in extent, the radio counterparts of the jet and counterjet^{3,4}. When observed with a resolution of $\approx 5''$, the structure of these outer components is similar and depends little on observing frequency, but at higher resolutions the jet component is seen to possess fine structure which the other lacks^{5,6}. Here I report results of interferometer observations at 408 and 1,666 MHz, with resolving powers $\approx 0.5''$ along the jet, which reveal the position and extent of this fine structure in more detail.

At 408 MHz the interferometer was formed from the Mk IA (76 m) telescope at Jodrell Bank and a 25-m paraboloid at RRE Defford (baseline 172,700 λ). At 1,666 MHz the Mk II (38 m $\times$ 25 m) telescope and the Mk III (38 m $\times$ 25 m) telescope were

Table 1 Parameters of the models fitted to the observations of M87

a 1,666 MHz	Component separation from nucleus ($''$)	Position angle (degree)	Half-power length ($''$)	Half-power width ($''$)	Position angle of elongation (degree)	Flux density (Jy)
	0.0*		U*	U*		3.5*
	0.0*		0.6 $\pm$ 0.3	U*	300 $\pm$ 30	1.5 $\pm$ 0.5
	9.1 $\pm$ 3.0	289.6 $\pm$ 3.0	5.5 $\pm$ 3.0	U*	292 $\pm$ 5	1.8 $\pm$ 0.6
	12.5 $\pm$ 0.3	290.5 $\pm$ 0.3	1.5 $\pm$ 0.3	U*	288 $\pm$ 10	2.8 $\pm$ 0.5
	14.4 $\pm$ 0.2	289.6 $\pm$ 0.3	1.4 $\pm$ 0.3	U*	282 $\pm$ 10	1.4 $\pm$ 0.3
	17.6 $\pm$ 0.2	292.0 $\pm$ 0.5	2.3 $\pm$ 0.3	U*	298 $\pm$ 10	1.5 $\pm$ 0.3
b 408 MHz						
	0.0*		0.5 $\pm$ 0.2	U*	260 $\pm$ 20	4.6 $\pm$ 0.5
	8.2 $\pm$ 0.4	291.6 $\pm$ 0.4	1.8 $\pm$ 0.4	U*	304 $\pm$ 10	1.6 $\pm$ 0.5
	12.3 $\pm$ 2.0	287.0 $\pm$ 4.0	9.0 $\pm$ 3.0	U*	300 $\pm$ 20	2.7 $\pm$ 1.0
	12.3 $\pm$ 0.3	290.7 $\pm$ 0.5	$\leq$ 0.5	U*		1.0 $\pm$ 0.2
	14.5 $\pm$ 0.2	289.6 $\pm$ 0.3	0.8 $\pm$ 0.3	U*	290 $\pm$ 15	0.8 $\pm$ 0.2
	17.6 $\pm$ 0.6	291.6 $\pm$ 0.4	1.6 $\pm$ 0.3	U*	260 $\pm$ 15	0.7 $\pm$ 0.2

* Held constant during the analysis.

U, Unresolved, dimension arbitrarily put to zero.

used (baseline 132,300 λ). The 1,666-MHz interferometer incorporated an improved radio link system (Spencer and Warwick, unpublished) which compensates for changes in the link path delay due to changes in atmospheric conditions. The resulting phase stability enables the position of the radio nucleus to be measured; it is within 0.5'' in right ascension and 2'' in declination of the optical nucleus⁷, in agreement with earlier radio position measurements⁸⁻¹⁰.

Strip distributions in right ascension, obtained by direct Fourier inversion of the 1,666 MHz amplitude and phase data,

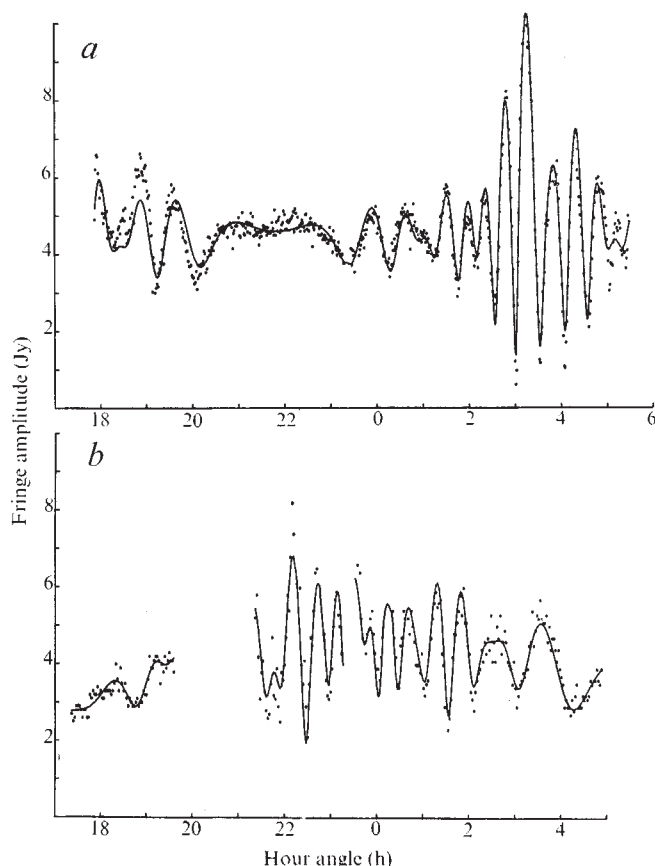


Fig. 1 Variation of fringe visibility with hour angle at a, 1,666 MHz; b, 408 MHz.

show that there are other compact components in the source. The two which are best defined lie on the jet side at between 12'' and 15'' and at $\approx$ 18'' away from the nucleus. On the other side the amount of emission from compact components is smaller but there may be weak features ($\lesssim$ 0.5 Jy) at $\approx$ 8'' and $\approx$ 13'' away from the nucleus. To investigate the radio structure further the fringe amplitude data were analysed using a model-fitting technique which assumes that the components have Gaussian symmetry. The data are shown in Fig. 1a with the curve which would be given by the adopted model brightness distribution. Because the range of resolutions in the N-S direction is poor, at the declination of M87, the widths of the components are not well defined. In the adopted model all the widths were constrained to be zero but values up to $\approx$ 2'' are also consistent with the data. The values of the optimised parameters of the model are given in Table 1.

The 408-MHz data were only analysed using the model-fitting technique and the adopted values of the parameters are also given in Table 1. The absolute positions of the components cannot be determined at this frequency but from the agreement between the two models it is clear that the 408-MHz observations refer to the same regions of the source as those at 1,666 MHz and that the most intense component is in fact the radio nucleus.

The spectra of the individual sources in the nucleus cannot be determined unambiguously. Graham's attempt³ is incorrect because he recognised only two components and he also included some flux density measurements which are almost certainly contaminated by contributions from compact components in the jet. The present work shows that at least one of the very compact nuclear sources must have a low frequency cut-off near 1 GHz, for the unresolved component in the model has a flux density of 3.5 Jy at 1,666 MHz but less than $\approx$ 2 Jy at 408 MHz. Further high resolution observations are needed to delineate the spectra more clearly.

The agreement between the well defined components of the 408 and 1,666 MHz models means that the basic structure of the radio jet depends little on observing frequency. The other components in the two models do differ but at both frequencies they indicate that the region between the nucleus and the well defined components in the jet is also a source of radio emission. The brightness distribution of this region is probably complex and synthesis observations are required to reveal its structure unambiguously. Figure 2 shows a photograph of the central region of M87 (ref. 11) and below, plotted on the same scale, contours of the radio emission as described by the model at 1,666 MHz. The striking correspondence between the brightness distributions at optical and radio wavelengths indicates that the structure of the jet is essentially the same over a frequency range of $\approx$ 10⁶:1.

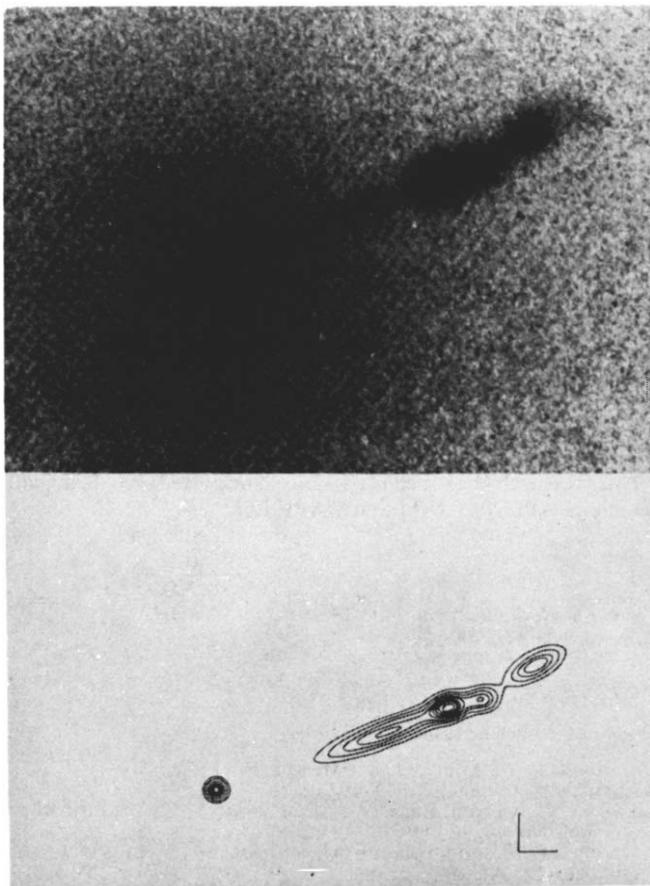


Fig. 2 Comparison of the optical and radio structures of the M87 jet. The photograph is taken from Felten *et al.*¹¹. North is at the top and east to the left. Many contours have been omitted from the intense nuclear source for the sake of clarity and the poorly defined component widths have been arbitrarily assigned the value 1''. The L in the bottom right hand corner represents 2'' in each coordinate. (Photograph reproduced with permission of the *Astrophysical Journal*.)

A convincing explanation of the physics of the M87 jet is still awaited. The present results show clearly that the electrons responsible for the radio and optical synchrotron emission have very similar spatial distributions, but further high resolution work, especially to locate the variable component¹² and to determine the polarisation structure of the knots at different frequencies, is required before the radio observations can place important constraints on theories of the jet.

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22.2-GHz high emission nebulosities associated with the Carina Nebula central region

MAPS of Carina Nebula, obtained at relatively low frequencies, have shown that most of the radiation arises from a central region and has a simple double structure, separated by about 9.4'. Gardner *et al.*¹ studied Carina Nebula at 5 GHz, the highest frequency so far used, with the Parkes radio telescope and observed a beamwidth of 4'.

Shaver and Goss², used the Molonglo interferometer at 408 MHz (with 2.65' and 2.86' beams in right-ascension and declination, respectively) to study the same region. The two main sources, G287.4–0.6 and G287.6–0.6 (Car I and Car II, respectively¹) were suggested as optically-thin thermal regions in the range 408 MHz to 5 GHz, and were assigned electron temperatures of 8,600 K and 8,100 K, respectively.

Using the 13.7 m radio telescope of the Itapetinga Radio Observatory, Atibaia, SP, Brazil, we derived a continuum map at 22.2 GHz from the central region of Carina Nebula, with a beamwidth of 4', and a linearly polarised feed. Because of the large angular extension of the source, we used a load-switching and on-off technique, with beams 45' apart, for the mapping. Successive positions measured in the source were separated by one beamwidth in declination and/or right-ascension. We corrected for tropospheric attenuation and calibrated all measurements against Virgo A, for which we

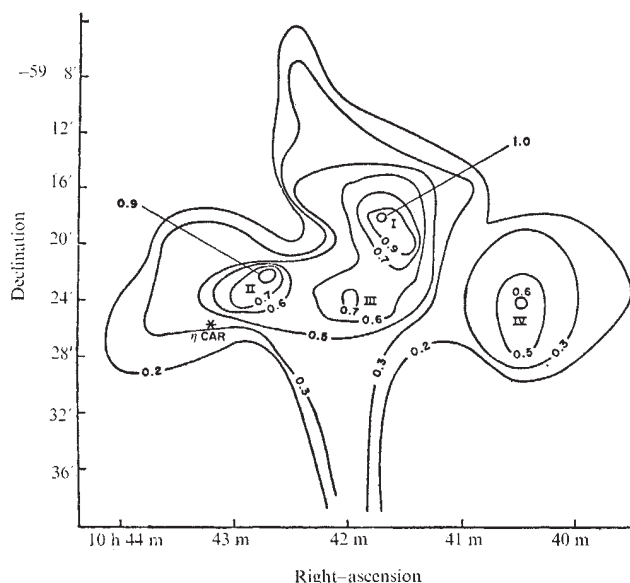


Fig. 1 A radio map from the central region of Carina Nebula obtained on a continuum at 22.2 GHz. Isotherms are normalised in relation to the stronger emission at Car I.

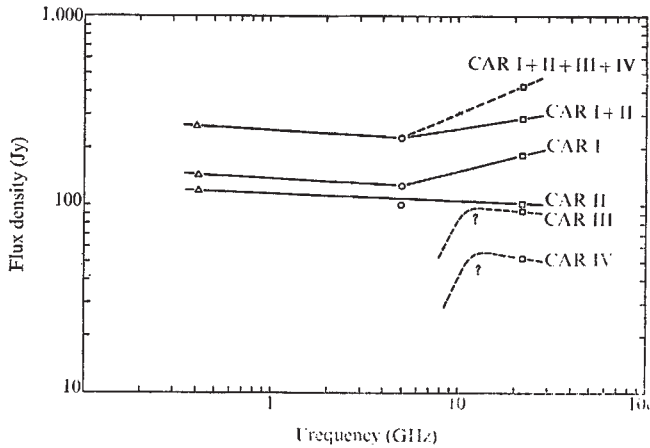


Fig. 2 Integrated spectra for the different components in Carina Nebula. At 22.2 GHz, the flux densities are: 183 Jy for Car I; 104 Jy for Car II; 94 Jy for Car III and 52 Jy for Car IV (all within $\pm 20\%$). Δ , ref. 2; $\circ$, ref. 3; $\square$, our data.

assumed a flux density of 21.5 Jy at 22.2 GHz. The inaccuracies of the intensities measured from Carina Nebula were estimated to be about $\pm 20\%$, as the sum of r.m.s. errors, attenuation errors, and source size correction errors. The absolute pointing accuracy of the radio telescope is known to be better than $20''$, which is negligible compared with the beamwidth at 22.2 GHz.

Figure 1 shows the map at 22.2 GHz, not restored for aerial smoothing. The two main sources identified at 5 GHz^{1,3} agreed well with those at 22.2 GHz, but the whole region became rather more complex at this higher frequency. Two new structures appeared, one at about $\alpha_{1950.0} = 10^h 42.0^m$ and $\sigma_{1950.0} = -59^\circ 24.0'$, and another at $\alpha_{1950.0} = 10^h 40.4^m$ and $\sigma_{1950.0} = -59^\circ 24.0'$ (corresponding to G287.5-0.7 and G287.2-0.8, respectively) which we have designated Car III and Car IV, respectively.

No enhanced radiation was measured in the direction of η Car itself, and the radio emission from the central region seemed to follow southwards in a straight bridge centred at about $\alpha_{1950.0} = 10^h 42.0^m$. There is generally no good correspondence between the radio features at 22.2 GHz and the optical features from the nebula.

Figure 2 shows spectra for the central region of the nebula, using data from Gardner and Morimoto³, and Shaver and Goss². We restored the sizes of the sources by simplified techniques^{4,5}; to derive integrated fluxes at 22.2 GHz and we assumed a Gaussian form for the antenna beam, and Gaussian distribution of brightness across the sources, with elliptical symmetry. Only Car II seemed to be a normal H II region, optically thin in the range 408 MHz to 22.2 GHz. The other spectra, the integrated ones, and that one for Car I, seem to receive contributions from sources with high emissions, still optically thick at 5 GHz, and probably thin at 22.2 GHz.

The two identified and resolved new sources, Car III and Car IV may be very compact H II regions, with turnover-frequencies in the range 5 to 22.2 GHz. If we assume that these sources are already optically thin at 22.2 GHz, and the turnover frequency f_c is about 10 GHz, we can estimate a mean emission after Terzian and Dickey⁶

$$\langle E \rangle = (f_c^2 T_e^{3/2}) / k_e \quad (1)$$

where T_e is the electron temperature, and the absorption coefficient k_e at the turnover frequency (MHz) is

$$k_e = 9.8 \times 10^{-15} \ln(49.5 T_e^{3/2}) / F_e \quad (2)$$

Assuming that $T_e \approx 8,600\text{K}$ for these sources, we obtain high mean emissions, of about $4.3 \times 10^8 \text{ cm}^{-6} \text{ pc}$.

An accepted distance of Carina Nebula is about 2.7 kpc (ref. 7), from which we estimate an approximate radius for Car IV (better defined in Fig. 1) of about 3.2 pc. The value for the emission is

$$\int_0^R N^2 dR \quad (3)$$

where R is the radius and N the electron density. For a constant distribution of N along R , and along the line of vision, we find an electron density of about $1.2 \times 10^4 \text{ cm}^{-3}$. The values for T_e , $\langle E \rangle$ and N , estimated for the enhanced sources in the central region of Carina Nebula, correspond to an extreme case of a typical compact H II region as proposed by Mezger⁸. Measurements at a frequency between 5 and 22.2 GHz, and at a frequency above 22.2 GHz, are required to understand better the nature of this interesting nebula.

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Gyron Field—gravitational analogue of magnetic force

AN electrostatic field E in a stationary coordinate system, when referred to a moving coordinate system, can produce both an electric field E^* , and a magnetic field B^* . If v is the uniform velocity of motion, perpendicular to E , then by the usual Lorentz transformation:

$$E^* = E / \sqrt{1 - \beta^2} \text{ and } B^* = v \times E / c \sqrt{1 - \beta^2}, \quad (1)$$

where $\beta = v/c$.

Consider two stationary point masses, m_1 and m_2 , with respective electric charges q_1 and q_2 . Suppose that the particles are located at $0, r/2, 0$ and $0, -r/2, 0$ in a system of Cartesian coordinates. If the masses and charges are chosen so that the force of electrostatic repulsion exactly balances that of gravitational attraction, then

$$q_1 q_2 / 4\pi\epsilon_0 r^2 - G m_1 m_2 / r^2 = 0 \quad (2)$$

where G is the constant of gravitation and ϵ_0 is the electrical permittivity of space. Positive signs indicate repulsion and negative signs attraction.

Now suppose that the two particles, while maintaining the same distance apart, are moving with uniform velocity, v ,

parallel to the x axis. This motion will not disturb the equilibrium of the particles.

From equation (1), the electric force of particle 1 acting on particle 2 becomes

$$\mathbf{E}^* q_2 = q_1 q_2 / 4\pi\epsilon_0 r^2 \sqrt{1-\beta^2} \quad (3)$$

Similarly, the magnetic force becomes:

$$\begin{aligned} q_2(\mathbf{v} \times \mathbf{B}^*)/c &= -(q_2/c^2) \mathbf{v} \times (\mathbf{v} \times \mathbf{E}) \\ &= -q_1 q_2 \beta^2 \mathbf{r} / 4\pi\epsilon_0 r^3 \sqrt{1-\beta^2} \end{aligned} \quad (4)$$

The negative sign indicates that this component of force represents attraction.

The electric charges q_1 and q_2 are invariant under a Lorentz transformation, whereas the masses m_1 and m_2 must vary as

$$m_1^* = m_1 / \sqrt{1-\beta^2}, \text{ and so on} \quad (5)$$

Within the framework of special relativity, the ordinary gravitational force, analogous to equation (3) in the moving system, becomes:

$$Gm_1^* m_2^* / r^2 \sqrt{1-\beta^2} = Gm_1 m_2 / r^2 (1-\beta^2)^{3/2} \quad (6)$$

Hypothetically, linear motion of translation will not disturb the equilibrium of the two point masses. The gravitational force in equation (6) is, however, not functionally compatible with the total electric forces of equations (3) and (4). To achieve the balance of forces a velocity-dependent gravitational force analogous to the magnetic component of equation (4) (which is proportional to β^2) must be added to equation (6). Thus:

$$[q_1 q_2 / 4\pi\epsilon_0 r^2 \sqrt{1-\beta^2}](1-\beta^2) - [Gm_1 m_2 / r^2 (1-\beta^2)^{3/2}] (1-A\beta^2) = 0 \quad (7)$$

where A is a parameter to be determined.
From equation (2):

$$A\beta^3 = 1 - (1-\beta^2)^2;$$

or

$$A = 2 - \beta^2 \quad (8)$$

This analysis seems to establish the existence of a gravitational force dependent on the relative velocities of moving bodies, as well as on their masses. We suggest that this special force, which must apply to translational motion as well as to rotational motion, be called the 'Gyron Force'.

Lorrain and Corson¹ have mentioned the possibility that such a force exists. They have speculated about "a gravitational equivalent of the magnetic field" but have given no quantitative statement. Moreover, they indicated that this force "could only be attractive", whereas the positive sign of A in equation (7) indicates that the force, as we have derived it, is repulsive. Furthermore, Blokland² has concluded, by somewhat obscure reasoning, that the quantity we have designated as A should probably have a value of about 2.

In special relativity, the apparent mass of a body depends on its energy, in terms of the familiar Lorentz transformation. Precise experiments³ have shown that gravitational and inertial masses are identical to at least 13 significant figures. Apparent mass rather than rest mass must, therefore, be used to define gravitational forces. Thus, our derivation of the gyron field disagrees with that of Sciama⁴ who used the rest mass instead.

This analysis ignores such phenomena of general relativity as curvature of space. It is, however, clear that the principle must apply in the area common to both general and special relativity. We recognise that a complete derivation must be built on a tensor rather than a vector or scalar theory of gravitation. The result derived here does not depend, however, on any specific theory of gravitation.

We further point out that the existence of forces associated with moving or, especially, rotating masses strongly supports the idea that gravitational waves exist. Such waves must be quadrupole in character. Only the existence of electric charges of opposite sign makes possible the radiation of dipole electromagnetic waves.

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Observation of Comet Kohoutek at 1.4 mm

We made observations of Comet Kohoutek at Kitt Peak with the National Radio Astronomy Observatory (NRAO) 36-foot telescope during two consecutive periods: December 27, 1973–January 2, 1974; and January 6–January 13, 1974.

We used a 1-mm system with our own high performance three-part bolometer¹: diameter=6.5 mm; $\tau \approx 7$ ms; optical NEP at 1 mm = 5×10^{-14} W Hz⁻¹. It has a limit of detection of 2 Jy per hour of observation on the radiotelescope with good weather. The system applies a differential modulation at 20 Hz on the sky with two beams each of 1' separated by 2.2'. Scanning of the source and secondary low-frequency modulation to eliminate drifts is carried out by moving the whole telescope.

The bandpass of the system ranges from 1 mm to 3 mm, with a sharp cutoff at 1 mm; the efficiency increases from about 25% at 1.2 mm to 100% at 2 mm. The mean wavelength on a planet of spectral index 2 is thus about 1.6 mm. In the case of cometary dust emission, with a spectral index of pro-

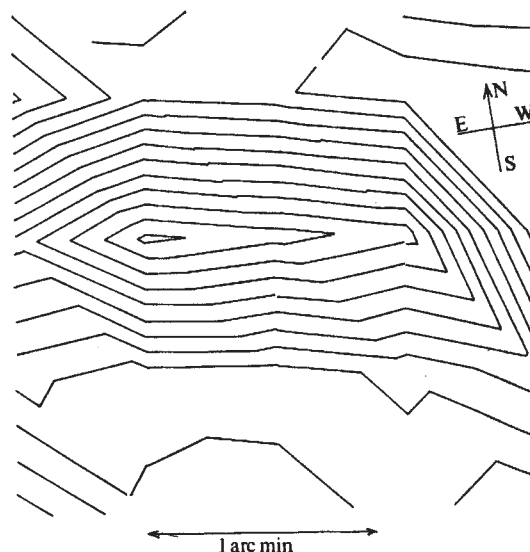


Fig. 1 Comet Kohoutek December 30, 1973; differential isoflux at 1 mm. This map shows interpolated values of points separated by 0.5'. It is obtained from preliminary data taken from 1855 to 2000 UT, which are not corrected for field rotation or for atmospheric drift.

bably between 3 and 4, we estimate the mean wavelength to be 1.4 mm.

The field of view of the instrument was of the order of $1'$ quasicircular, determined from observations of unresolved sources such as planets. The absolute calibration of the flux was made through a straightforward comparison between the observed cometary emission and the flux coming from different planets, observed through the dome during the same day (J.D.G.R. *et al.*, unpublished), corrected for the atmospheric attenuation given by the relationship $\exp(-a \sec z)$, where a is a function of time and z is the zenith angle. The position of Venus and Jupiter very close to the comet made this calibration particularly easy.

During the first period, Comet Kohoutek (1973f) was detected clearly on December 30, 1973. On the first evaluation, the cometary flux was of the order of 100 ± 30 Jy. It was even possible to map the comet's head (Fig. 1). In spite of the poor spatial resolution, it is quite clear that this shape is elongated and orientated in the direction of the Sun, demonstrating that it was the comet, which was under observation.

We next observed the comet on January 12, 1974, when it was much fainter: its flux was then of the order of 10 ± 5 Jy. This result, compared with the first, shows that to a first approximation the cometary flux at a wavelength of about 1.4 mm seems to decrease as r^{-2} , where r is the distance from the comet to the Sun.

The total energy available for heating the dust grains is given by:

$$E_t = \sigma T^4 \sim 1/r^2$$

Therefore, the temperature of the grains should change as $r^{-1/2}$. The observed decrease in flux density is much more rapid than anticipated, implying a decrease in the global emissivity of the dust cloud.

This decreasing rate of emissivity may arise from several sources. Two likely possibilities are, first, a change of the dimensions of the emitting head (that is, a probable change in the production rate of dust); second, a change in the emissivity of the dust grains.

Consider the first hypothesis, and suppose that the flux should change as $\Delta^{-2}r^{-1/2}$, where Δ represents the distance between the comet and Earth. It can then be calculated, to fit the observed variation ($\Delta^{-2}r^{-2}$) to the predicted variation, that the size of the head changed at that wavelength from roughly $1'$ at the end of December to about $20''$ around January 12, 1974.

This observation, which represents the first detection at 1.4 mm of a cometary emission, provides new information concerning the rate of dust production and the ejection mechanism of the dust. Comparison of this observed flux with others made at shorter and longer wavelengths, using the black-body emission law, will probably give the dependence on wavelength of dust grain emissivity, which is directly related to their composition and size.

We wish to thank Elisabeth Rather for programming assistance and the NRAO team for their collaboration at Kitt Peak National Observatory. These observations were sponsored by Centre National de la Recherche Scientifique, Centre National d'Etudes Spatiales, and Institut National d'Astronomie et de Géophysique.

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Microwave search for molecules in Comet Kohoutek

WE report here a molecular radio line search in Comet Kohoutek using the 108–116 GHz spectral line receiver on the NRAO 36-foot telescope during January 14–18, 1974. We discuss some of the reasons for the negative results obtained in our search for transitions of methyl cyanide, cyanogen, cyanoacetylene, propynal and formic acid (all good 'parent' molecules for the diatomics and radicals known in the optical spectra of comets).

We particularly searched for the two transitions of methyl cyanide which had been detected by Ulich and Conklin¹ when the comet was 0.8 AU from the Sun before perihelion. As we were observing the comet after perihelion, when it was approximately the same distance from the Sun, the temperature of the cometary material and the excitation conditions should have been comparable. The transitions were detected at the level $T_A \sim 0.6 \pm 0.1$ K, and our peak-to-peak upper bound for the transitions was $T_A \sim 0.3$ K.

Initially, we thought that this might indicate a time dependence in the sublimation of 'parent' molecules, perhaps caused by inhomogeneities in the composition of the comet nucleus. We have since learned, however (T. Clark, private communication) that there was a 'time of flight' error in the position ephemeris of the comet that we and many other radio-astronomers used. During our observations, the magnitude of the error was $\sim 45''$, as it was during the observations of Ulich and Conklin who used the same ephemeris. For observations with beamwidth $1'$, a pointing error of this magnitude is clearly very serious. It seems unwarranted therefore to draw many conclusions about the existence of molecules in the comet from these observations.

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Solar neutrino puzzle

DAVIS and his collaborators, using a technique based on the reaction $^{37}\text{Cl}(\nu, e^-)^{37}\text{Ar}$, have been able to set a bound to the flux of neutrinos arriving from the Sun. The latest experimental results¹ correspond to 1 SNU (solar neutrino unit, 1×10^{-36} neutrino capture per ^{37}Cl nucleus per second), whereas solar models yield values higher by one order of magnitude². Here I propose an explanation of the discrepancy between theory and experiment on the basis of the assumption that strong interactions between nuclear particles are of gravitational nature³⁻⁶.

I shall assume that within the range of nuclear forces, the gravitational constant is 38 orders of magnitude larger. For

distances larger than the Compton wavelength of the proton $\lambda = 2.1 \times 10^{-14}$ cm, its value decreases exponentially, not to zero, but rather to a value of the gravitational constant as we know it. On the other hand, for distances within 0.21 fermi, the gravitational force would behave as a short range, charge-independent, non-exchange force, responsible for the strong interactions between nucleons. To remain in agreement with the mass defects of atomic nuclei, the value of the gravitational constant at distances which are small compared to the wavelength of the proton has to be of the order $G^* = 2.75 \times 10^{30}$ c.g.s.

The assumption of a limiting range in the gravitational potential is in itself not as arbitrary as it may seem. The Hubble diagram of quasars⁷ suggests that the cosmological constant is not zero, which represents by definition a limiting range of the gravitational field. I suggest that there is also a lower boundary. Our physical world is observable between $\sim 4,000$ Mpc and $1/5$ fm.

Should strong interactions between nucleons be gravitational, then all signal carriers with zero rest mass, such as photons and neutrinos, would interact with nucleons like hadrons if their energy is a few GeV. This has been observed for photons in experiments at DESI, Hamburg. An increase of the interaction forces with increasing gravitational mass of the interacting particles could also explain the unexpected increase in the total proton-proton cross section obtained with the CERN intersecting storage rings by the Pisa and Stony Brook teams at energies above 20 GeV.

At $G^* = 2.75 \times 10^{30}$ c.g.s., the gravitational radius of the proton will become of the order of the Compton wavelength of the proton, leading to the inference that elementary particles behave as black holes. A hypothetical observer within such a black hole can use the Friedmann field equations. Cosmological entities observable within this 'lilliputian' universe have nuclear physical counterparts for an outside observer. For instance, the absolute value of the cosmological constant within the lilliputian universe is identical with the Compton wavelength of the particle. Because of the large value of G^* , the proton will occupy a hypersphere of radius $\sim \lambda$ embedded, as is our world, in a four-dimensional continuum. Should the hypersphere of the proton intersect the hypersphere of our world, a proton will appear in our world, as a closed two-dimensional space of constant curvature. (Antiprotons intersect our three-dimensional space from the other side.) Assuming that the hyperspheres of particles cannot intersect each other, we arrive at the concept of the hard core, with a range of $\sim 2\lambda = 0.42 \times 10^{-13}$ cm, which is about the range of the hard core, 0.53×10^{-13} cm, in Levy's potential of nuclear forces. Cosmological, quantum mechanical laws and geometrical rules of two- to four-dimensional spaces supply a sufficient number of equations to yield a quantitative model-like description of fundamental elementary particles³⁻⁵.

A gravitational interaction between neutrinos and nucleons leads to the inference that even radioactive neutrinos, or neutrinos produced in nuclear reactions occurring in the interiors of stars, can transfer a momentum to nucleons and lose energy while passing through the mass of the star. As long as the transferred energy is small compared to the rest mass energy of the target particle, the momentum transfer can be computed in a classical manner. A neutrino of relativistic mass E_ν/c^2 , passing at a distance a from a nucleus of mass m , will through gravitational interactions transfer a momentum:

$$p_a = aE_\nu 2G^* m \lambda^{-1} c^{-3} \int_a^\infty r^2 (r^2 - a^2)^{-1/2} (1 - r) \exp(-r) dr$$

where distances are expressed in units of the Compton wavelength of the proton, $\lambda = 2.1 \times 10^{-14}$ cm. The energy loss of the neutrino will be

$$\Delta E = p_a^2 / 2m \text{ erg}$$

This energy loss, occurring in an interaction with a nucleus of atomic weight A , can be approximated by

$$\begin{aligned} \Delta E &= 1,630 A E_\nu^2 \exp(-4.10a) & 0.5 < a < 1.0 \\ &= 390 A E_\nu^2 \exp(-2.66a) & 1 < a < 5 \end{aligned}$$

Assuming that in such elastic interactions a neutrino cannot approach closer to a proton than the hard core range $a = 2r_0 = 0.625$, and energy losses at impact parameter $a > 5$ are negligible, the energy loss suffered by a neutrino of energy E_0 MeV after passing through an absorber of unit (1 g cm^{-2}) thickness will be

$$dE/dR = 8.7 \times 10^{-6} E_\nu^2 \text{ MeV}$$

After traversing an absorber of $R \text{ g cm}^{-2}$ thickness a similar neutrino will emerge with an energy

$$E_\nu = E_0 / (8.7 \times 10^{-6} R E_0 + 1) \text{ MeV}$$

I note that this result is insensitive to the chemical composition of the absorber. (Inelastic collisions are not considered.)

The average density of the Sun being 1.41 g cm^{-3} , and its radius 5.4×10^{10} cm, $R = 7.6 \times 10^{10} \text{ g cm}^{-2}$. A neutrino starting with an energy E_0 MeV at the centre of the Sun will leave it with energy $E = E_0 / (6.57 \times 10^3 E_0 + 1) \text{ MeV}$. A neutrino from either the ${}^7\text{Be}$ reaction ($E_0 = 0.861 \text{ MeV}$), or the ${}^8\text{B}$ reaction ($E_0 = 14.06 \text{ MeV}$) would arrive at the Earth with energy $\sim 150 \text{ eV}$, far below the threshold (0.81 MeV) needed to detect neutrinos in the Homestake mine experiments of Davis.

The energy loss would, however, be much less if the neutrinos were to originate in sunspots, or in stars with densities much below the density of the Sun. One would expect Davis to observe some neutrino flux through the ${}^{37}\text{Cl}(\nu, e^-){}^{37}\text{Ar}$ reaction during sunspot activity, or perhaps during outbursts of nearby supernovae (see ref. 8 for example).

My hypothesis could be tested at an accelerator where neutrinos and antineutrinos of energy 0.5–5 GeV are produced. According to my ideas, some of their energy would be lost in the iron shield used to absorb the muons and other ionising particles. A neutrino of energy 1 GeV, after traversing an iron absorber 20 m thick, would emerge with an energy of 700 MeV, adequate to produce muons when entering a bubble chamber.

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Elemental abundances in interplanetary dust

It is generally believed that the main fraction of dust in the interplanetary medium is generated by the gradual disintegration of short-period comets in the inner Solar System^{1,2}. Analysis of interplanetary dust-grains is, accordingly, a technique for gathering information on the nature of cometary matter. Until now, however, no particle with

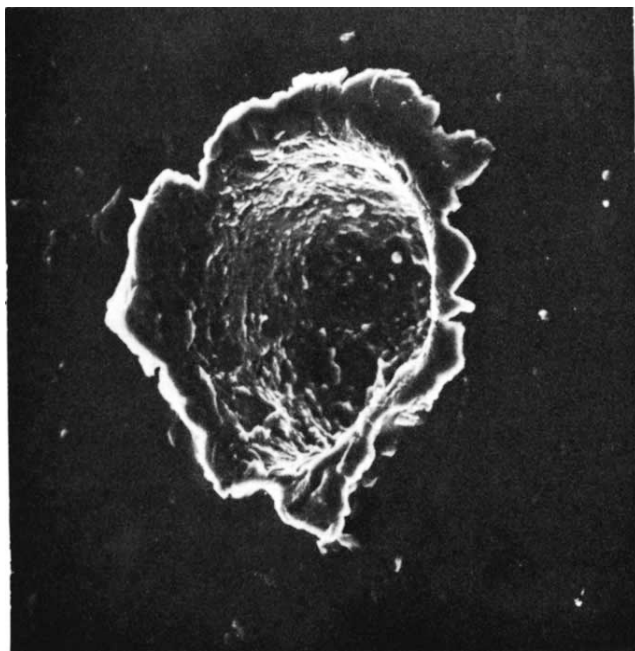


Fig. 1 110 μm diameter micrometeorite crater from Skylab-IV.

an unarguable interplanetary origin has been subjected to detailed chemical analysis.

Here we report the measurement of elemental abundances in two interplanetary dust grains obtained by analysis of meteoroidal residue found inside micrometeoritic craters. The two craters were discovered by scanning optically the 800 cm^2 aluminium surface of the S-228 transuranic cosmic-ray experiment, exposed to space for 67 d during the Skylab-IV mission. The larger crater is 110 μm wide (measured at the intersection of the pit with the plane of the original surface) and 75 μm deep (measured from the pit bottom to the original surface plane). The smaller crater is 35 μm wide and 26 μm deep. The morphology of both craters is identical to hypervelocity craters produced in laboratory simulations and to craters found on metal grains from lunar soils³. The morphology is diagnostic proof that the craters were produced by genuine interplanetary dust grains impacting at velocities in excess of a few km s^{-1} . Laboratory calibrations on aluminium targets (J. F. Vedder, private communication) indicate that the meteoroids had diameters of 30 μm and 9 μm .

In the scanning electron microscope, residue of the impacting meteoroids can be seen as conspicuous blebs and stringers lining the walls and floors of the craters (Figs 1 and 2). Energy-dispersive X-ray analysis of the 110 μm crater in the SEM revealed the presence of Fe, Mg, Si, Ca and Ni in the crater bottom with relative peak heights qualitatively similar to those obtained for chondritic meteorites. For a more quantitative result, both craters were analysed in an ARL-EMX electron microprobe. The craters were tilted with respect to the electron beam and spectrometer to eliminate blocking of the emitted X rays by the steep crater walls. The sizes of the spots analysed on the floors of the large and small craters were 30 μm and 3 μm respectively. Only a single spectrometer was used for all elements, thereby eliminating the differential geometry problems that would arise from using different spectrometers for different elements. During an analysis run, the crater was kept in a fixed position and the spectrometer was scanned over all of the elements analysed. Before and

after the analysis runs, the spectrometer was scanned over mineral standards for calibration. Repeatability of the peak measurements on the standards was good, and errors arising from spectrometer tuning were small compared with errors caused by the geometry of the crater bottom. Two runs were carried out on each crater. The beam was repositioned and refocused between runs. The aluminium substrate material round the craters was pure; the only indigenous elements detected in it were Ti (130 parts per million), Mg (300 p.p.m.) and Si (4,000 p.p.m.). The only significant substrate interference was by Ti. The Ti upper limit in Fig. 3 represents the substrate Ti concentration.

Because of the complex geometry of the crater it was not possible to use conventional correction procedures on the probe data; the results should accordingly be considered as only semi-quantitative. The geometry affects the absolute X-ray flux received by the spectrometer but does not affect strongly the relative intensities from different elements. The

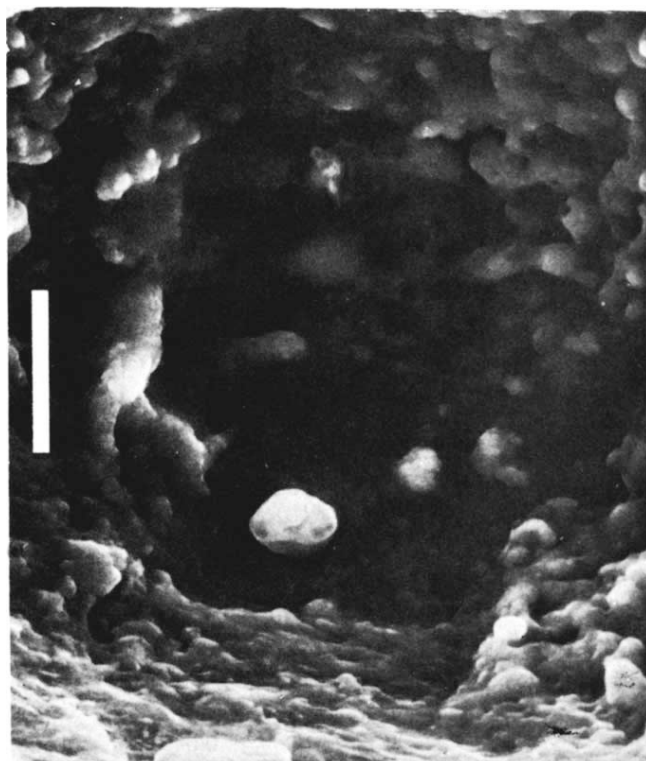


Fig. 2 Bottom of the 110 μm crater showing physical evidence for meteoroid residue. Scale bar = 10 μm .

measured relative abundance ratios should be fairly accurate. This ratio technique has been applied with considerable success^{4,5} to the analysis of other specimens with non-planar geometry.

The small crater was analysed with a point beam at several spots around the crater floor. Iron and sulphur were detected as major elements, and Ni and Mg as trace elements. The small size of the crater makes quantitative analysis difficult but the implied Fe:S ratio is roughly consistent with FeS. The Fe, S and Ni residues indicate that the crater may have been produced by a troilite (or pyrrhotite) meteoroid. Troilite is a common mineral in most meteoritic types. The Fe:Mg ratio was 50:1, so the Mg content is not of major concern.

The results of the two analyses on the large crater are shown in Fig. 3. The two runs agree well with each other, and the agreement with carbonaceous chondrites is within a factor of two for the seven elements quantitatively analysed. Sulphur, a cosmically abundant but volatile element, is not plotted because it was not looked for during that analysis. A sulphur analysis done at a later date, however, showed that sulphur is present in the crater with quite high abundance, qualitatively similar to the abundances of the Fe, Mg, Si group, and compatible with chondritic abundances.

The crater analyses indicate that, for two randomly sampled meteoroids, a 9 μm particle had a composition consistent with troilite and a 30 μm particle contained chondritic abundances. Particles of similar size and chemistry are common in carbonaceous chondrite meteorites and the implication is that the source material for interplanetary dust may be similar to this primitive meteoritic type. Evidence for a similarity to carbonaceous chondrites rather than to other meteorite types comes from the inferred grain sizes within the 30 μm particle. As no known mineral contains cosmic abundances, the particle must have been composed of an aggregate of smaller mineral grains. For the composition of the particle to be close to cosmic abundances the constituent grains must have been considerably smaller than 30 μm . Few chondritic meteorites outside the carbonaceous group have typical grain sizes as small.

Although these may be the first laboratory analyses of interplanetary grains, there are several other lines of evidence that support similarities between chondritic meteorites and interplanetary material. Studies of the extra-lunar component of lunar soils have demonstrated that the bulk of the micrometeoroid mass hitting the lunar surface has trace element abundances identical to C1 chondrites⁶. In addition

analyses of meteor spectra^{1,7} and measurement of mesospheric ion enrichment during meteor showers⁸ suggest that the bulk of millimetre-sized particles at 1 AU have abundances close to chondritic values. The apparent similarity between interplanetary material and carbonaceous chondrites should not, however, be construed as evidence that both types of object have a common source. The similarities are probably only a consequence of each type representing primitive, well preserved samples of early solar system materials.

From the microprobe analyses we estimate that some 10% of the two Skylab-IV meteoroids survived as residue. This degree of residue retention is consistent with laboratory simulations^{9,10} using iron projectiles, but not with studies of micrometeorite craters on lunar rocks¹¹. The difference may arise because the impact of Skylab was on soft metal whereas on lunar rocks it is normally on to silicate materials. The impact conditions are quite different in the two materials, as shown by laboratory observations that for a fixed projectile energy, a hypervelocity crater in metal will have almost ten times the volume of a crater produced in a silicate target¹⁰.

The successful detection of residues in the Skylab-IV micrometeoritic craters suggests that if other craters, in appropriate targets, can be recovered from space it may be possible to carry out a statistical evaluation of elemental abundances in debris generated by comets. Primitive meteorites contain large (> 30 μm) inclusions of materials with strongly non-cosmic abundances (such as olivine, magnetite, sulphides, calcium aluminium inclusions and chondrules). Residue analysis of 100 craters or so in the 100–300 μm size range would show whether or not these types of particles were also incorporated into the dust-ice matrix of comets. Positive or negative results of such a search would have fundamental implications regarding the interrelationships between comets and meteorite parent bodies.

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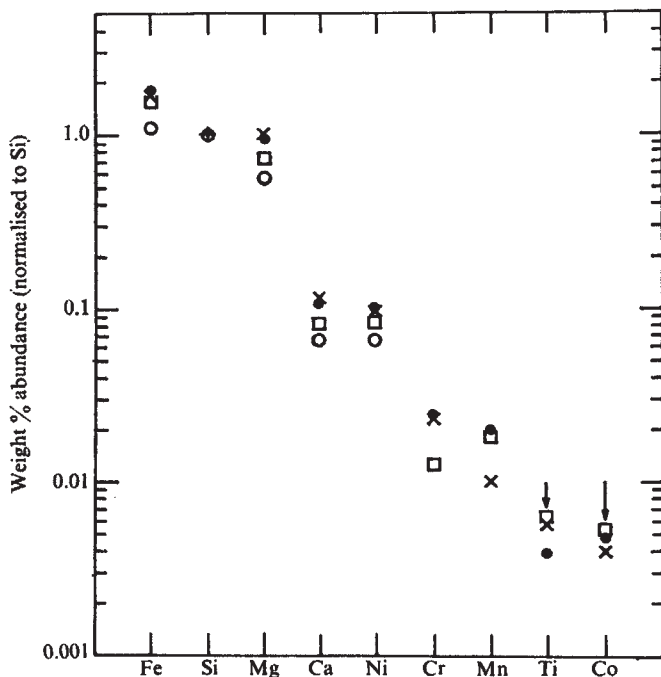


Fig. 3 Elemental abundances in the 110 μm Skylab-IV crater compared with average carbonaceous chondrite abundances. The open squares and open circles represent different probe runs on the crater. The filled circles and crosses are, respectively, C1 and C3 meteorite averages¹². Ti and Co were not detected in the crater and the upper limits are indicated by arrows.

Lunar magnetometry and mantle convection

THE hypothesis that the lunar mantle is at present convecting in a manner somewhat similar to that of the Earth is strongly supported by further analysis of lunar magnetometer data. Using the assumption that the electrical conductivity of the lunar interior varies with temperature, as laboratory measurements on rock-forming silicates indicate, I conclude that the Moon has a thermal boundary layer which is no more than 200 km thick and an interior which is relatively isothermal. Such a temperature profile is predicted by the hypothesis of a slowly convecting lunar mantle; it is in serious conflict with the hypothesis that the Moon is statically cooling and has a rigid crust extending to a depth of many hundreds of kilometres.

Early interpretation¹ of magnetic transient data from the lunar surface indicated the magnitude of the electrical conductivity of the lunar interior. Sonnett *et al.*² argued that these experiments should be interpreted in terms of important compositional differentiation about 200 km below the lunar surface to account for the large electrical conductivity peak there, which the data seemed to imply. Rama Murthy³ pointed out that such a conductivity profile could result from the presence of an FeS layer at this depth. Kuckes⁴ concluded that a serious question of uniqueness existed in the Sonett conductivity profile since the data could be fitted equally well with a simple two parameter profile. Subsequent computations by Sonett *et al.*⁵ confirmed this conclusion. Hobbs⁶ applied the Backus-Gilbert test to these two models and argued that the significance of the eight-layer model proposed by Sonett *et al.* was limited.

Because of the difficulty in assigning a unique conductivity profile to fit the observations, I have chosen to interpret these data in a somewhat different manner, which is much less sensitive to such difficulties. Since the electrical conductivity variation with temperature exhibited by most rock-forming silicates is very strong, direct comparison of temperature profiles with the experimental observations is much more inclined to be unique than are fits of the electrical conductivity profile. Though the exact chemical composition of the lunar interior is not known, the electrical conductivity σ can, with reasonable certainty, be assumed to have approximately the exponential variation with temperature T which characterises a semiconductor, namely, $\sigma = \sigma_0 \exp(-\epsilon/kT)$ (ϵ an activation energy, k Boltzmann's constant). Measurements of the average lunar density, the lunar moment of inertia and the composition of the lunar material returned to Earth suggest that the composition of most of the lunar interior is relatively uniform. I thus assumed, as a working hypothesis, that the important variation in the lunar electrical conductivity is due to temperature alone and can be written in the exponential form indicated, with the activation energy ϵ and the normalisation constant σ_0 fixed though unknown.

To abstract the information needed from the experimental data we continued the practice, initiated by Dyal and Parkin¹, of comparing in detail the behaviour of transients simultaneously recorded by Explorer 35 and Apollo 12. So that a large amount of data could be considered, individual transients were Fourier analysed in time and the ratio of the dominant amplitude was used to compute experimental values for the field amplification and the lunar transfer function A (ref. 7). The values of A derived by this method conform with those of Sonett *et al.*² in the domain of frequency overlap.

Figure 1 shows rather clearly that only the very outer portion of the Moon can have a significant temperature gradient. A significant temperature gradient extending deeper than about 200 km does not fit the data well.

The effect of varying the temperature gradient below 175 km, that is, below the knee in temperature profile 1, is shown in Fig. 2. The experimental predictions for these three temperature profiles were computed on the basis of the 2.33 eV activation energy reported by Duba *et al.*⁸ for olivine. Although all three temperature profiles plotted in Fig. 2 fit the data reasonably

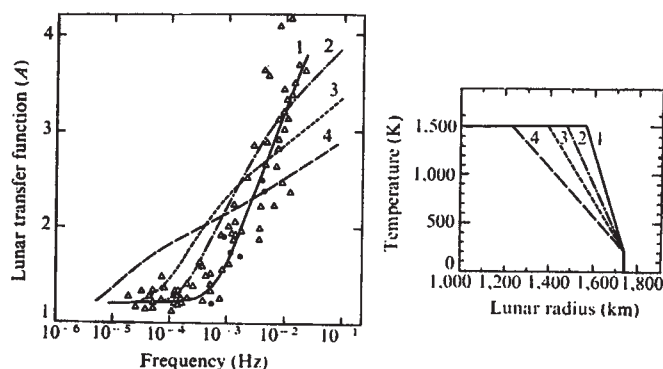


Fig. 1 Experimental values of the lunar transfer function compared to theoretical curves generated by several temperature profiles. The experimental points were obtained by simple Fourier analysis of magnetic transients in the Explorer 35 and Apollo 12 magnetometer records ●, From radial field night-time data; Δ, from horizontal field daytime data. The conductivity σ of the outer half of the Moon was assumed to depend on temperature as $\sigma = \sigma_0 \exp(-\epsilon/kT)$. ϵ was chosen to be 1.5 eV; the general results are not sensitive to the magnitude of ϵ if it is greater than about 1 eV. The temperature of 1,500 K for curve 1 was obtained by comparing the magnitude of the conductivity derived to the laboratory measurements of olivine by Duba *et al.*⁸. The theoretical curves include a 0.2 contribution to A by the diamagnetism of the solar wind²².

well, profiles with a temperature difference much greater than 400 K between 1,000 and 1,565 km radius clearly will not. Effectively, the steepness of the experimentally deduced variation of the transfer function A with frequency implies that the electrical conductivity cannot increase by more than an order of magnitude with depth between 1,565 and 1,000 km. Assuming that the significant conductivity dependence in the lunar interior is due to temperature and that the activation energy associated with this temperature dependence is large (as laboratory data indicate) the conclusion that the Moon has a thermal boundary layer which is only a few hundred kilometres or less thick is rather definite.

In the theoretical formulation of the transfer function A , the frequency ω and conductivity σ appear only as a product, that is, as $\omega\sigma$. Thus a change in the value of the conductivity normalisation constant σ_0 has the effect of only translating the curves for A horizontally in Figs 1 and 2. The shapes of the theoretical fits shown in Fig. 1 are not particularly sensitive to the value of the activation energy constant ϵ provided that it lies in the range to make the temperature dependence strong. For rock-forming silicates at the temperatures in question this assumption is fulfilled. The allowable temperature gradient in the interior region shown in Fig. 2 varies inversely with the activation energy ϵ . The vital features, required to obtain the conclusions derived, are that electrical conductivity increases rapidly with temperature, a lunar transfer function A which increases steeply with frequency to a value greater than 3, and the assumption of compositional uniformity.

Leavy and Madden⁹ have attempted to fit lunar magnetometer data using the temperature profile proposed by Toksoz *et al.*¹⁰ who considered the thermal history of a hot, cooling Moon. For present-day conditions they obtained a temperature gradient extending to a depth of more than 600 km. Leavy and Madden⁹ concluded that consistency with the magnetometer data can only be obtained by assuming that the conductivity of the lunar interior is characterised by a very low activation energy, that is, the conductivity does not change very rapidly with temperature. They point out that this property is difficult to reconcile with the measured properties of the materials generally believed to constitute the lunar interior.

The conclusion that the lunar interior below a depth of about 200 km or less is convecting, suggests a striking similarity to terrestrial conditions. The older parts of the Earth's lithosphere are about 100 km thick and have a temperature difference of about 1,300° C across them. About one half of the heat flow observed at most continental sites is derived from the mantle, that is, about 0.8×10^{-6} calorie $\text{cm}^{-2} \text{s}^{-1}$ (ref. 11). This value yields an average lithospheric thermal conductivity of about 8×10^{-3} calorie $\text{cm}^{-1} \text{°C}^{-1}$ and an average specific heat generation for the Earth's interior of 3.7×10^{-15} calorie $\text{cm}^{-3} \text{s}^{-1}$. This value for the thermal conductivity, a 200-km lunar lithosphere, and a temperature difference of 1,200° C across it yields a lunar mantle heat flow of 0.4×10^{-6} calorie $\text{cm}^{-2} \text{s}^{-1}$ and an average specific heat generation for the lunar interior of 7×10^{-15} calorie $\text{cm}^{-3} \text{s}^{-1}$. The heat flow computed is about one half that observed¹²; the specific heating is about double that for the Earth.

It is well known that the Earth's mantle convection induced linear features like mountains, oceanic trenches and island arcs, notably absent on the Moon. I note that the stress on a spherical shell due to loading by a linear feature with a fixed force length and angular width varies approximately as r/t^2 where r is the radius of the shell and t is its thickness. The force length associated with such a feature should vary in proportion to gravity if simple loading by a mountain range is in question, or in proportion to the radius of the body if the viscous drag of the circulating eddy in the lithosphere is involved (assuming constant viscosity). In either case the net stresses trying to break up the lunar crust would be about two orders of magnitude lower than on the Earth.

A great disparity apparently exists between the magnitude of the mantle electrical conductivity of the Moon and the Earth. Because of the complications introduced by the high electrolytic conductivity of the oceans and sediments, the electrical conductivity of the Earth's upper mantle is not well known; it is, however, apparently an order of magnitude greater than that of the Moon¹³. In addition to the very strong temperature effect the electrical conductivity is quite sensitive to composition, particularly changes in the Fe^{3+} concentration; thus, this disparity is perhaps not too surprising.

Turcotte *et al.*¹⁴ conclude that the critical temperature for mantle convection in the lunar interior is about 1,300° C which closely coincides with the temperature derived. Cassen and Reynolds¹⁵ have considered the effect of the temperature dependence on viscosity and have shown that convection can play an important role in the lunar thermal history. The thermal

models of McConnell and Gast¹⁶ which include convection are consistent with our conclusions. Tozer's convective model¹⁷ also predicts a temperature profile in agreement with our analysis; however, the temperature and radioactive heating rate he proposes seem rather low to obtain either convection or the observed value of the observed electrical conductivity.

Without convective transfer of heat most models of the lunar interior lead to the development of a thermal boundary layer too thick to be compatible with my analysis. Toksoz *et al.*¹⁰ have argued for a statically cooling Moon which, at present, has a thermal boundary layer about 600 km thick. Reynolds *et al.*¹⁸ have also shown that an initially cold Moon with a uniform carbonaceous chondritic composition develops a very thick boundary layer.

The computations of Hanks and Anderson¹⁹ show that a present-day thin thermal boundary layer consistent with our conclusion can, however, be generated by the proper combination of surface heat sources and initial temperature conditions at the time of lunar formation. Using a steady state approximation, their work shows that the assumption of complete early differentiation of the radioactive elements to the lunar surface can lead to a wide variety of thermal boundary layer thicknesses depending on the exact balance between initial temperature, absolute magnitude of the radioactive heating rate and the thickness of the heating layer. In any case, the temperatures they discuss are sufficient to make convection likely.

Urey²⁰ and Gold²¹ have long argued for a cold inactive lunar interior. Although exact calculations have not been made, T. Gold (private communication) has argued that deposition of the surface radioactive material somewhat after cold accretion could lead to general consistency with our results. Urey²⁰ and Gold²¹ have also raised concern about the support of the mascons by the lunar lithosphere. While the magnitude of the gravitational anomalies is, in terms of percentage, quite large compared to the terrestrial phenomena, Kaula²² has argued that isostatic compensation on the Moon is as good or somewhat better than on the Earth and that a 150-km thick lithosphere is sufficient to support the mascons.

I thank Drs Dyal, Parkin and Daily for their help. A discussion with Dr Ness on the role of plasma diamagnetism in the solar wind lead to clarifying this aspect of the work. Helpful discussions with Drs Bilson, Gold and Turcotte and computational help from Messrs Berkowitz and Beckwith are also acknowledged. This research was supported by a NASA grant.

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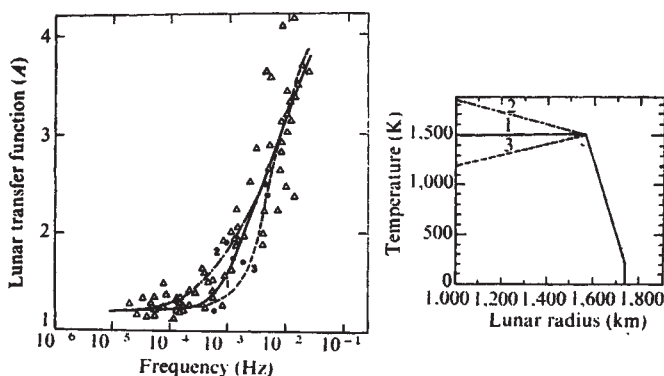


Fig. 2 The variation of the lunar transfer function as the temperature gradient in the lunar interior is varied. ●, From radial field night-time data; △, from horizontal field daytime data. The electrical conductivity was assumed to vary as $\sigma = \sigma_z \exp(-\epsilon/kT)$ with $\epsilon = 2.33$ eV. The magnitude of the temperature gradient in the interior region of profiles 2 and 3 is about 6% of that in the thermal boundary layer, that is, outside a radius of 1,560 km.

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Martian terminator waves

I HAVE suggested that the supersonic motion of the Earth's terminator could generate atmospheric waves¹, and Raitt and Clark claim to have discovered these waves in the terrestrial thermosphere². The Martian terminator will also be supersonic in a wide band of latitudes in the Martian upper atmosphere and may be expected to produce waves if the region's radiative response occurs on a shorter time scale than the dynamic response.

In Martian low and middle latitudes the terminator moves with a speed, V_T , of:

$$V_T = 240 \cos \phi \text{ m s}^{-1}$$

where ϕ is the geographical latitude. If we assume a pure CO₂ atmosphere then the speed of sound, C , is

$$C = 16.2 T^{1/2} \text{ m s}^{-1}$$

where T is the local atmospheric temperature. Supersonic waves may be generated at the equator in regions where $T < 220$ K, and at latitude 30° if $T < 164$ K. If we assume that the minimum temperature in the Martian atmosphere is 140 K at the tropopause³ then these waves can exist for $|\phi| < 37^\circ$.

In December 1971 Mariner 9 photographed Mars. There were 10 distinct observations of atmospheric wave structure on or near the terminator between latitudes +40° and -20°. Typical wavelengths were 40 km and the lack of any wave structure when photographed through orange filters indicates that the waves occurred in the atmospheric layer above the dust storms that were blowing during the Mariner expedition⁴. As the dust existed up to 10 km altitude it seems highly reasonable to assume that the terminator waves were generated in the stratosphere (that is, above 15 km) when the stratospheric temperature was sufficiently low. The observed latitudinal extent of the waves agrees well with this hypothesis. There is good reason to believe that the Martian stratosphere is isothermal over a height range of at least 15 km (ref. 3). Provided that the stratospheric temperature is low enough to generate supersonic waves then the resulting disturbances are in phase over that height range. The oscillation produced will, therefore, be evanescent, or very nearly so.

The only waves that will escape destructive interference are those for which the trace of the phase velocity in the direction parallel to the Equator equals the speed of the terminator within the source region of the wave. Only two types of atmospheric oscillations can have supersonic phase velocities^{5,6}. First, very low frequency infrasonic waves which satisfy the dispersion relationship

$$k^2 = \frac{\omega^2}{C^2} \frac{\omega^2 - \omega_{an}^2}{\omega^2 - \omega_B^2} \quad (1)$$

when they are evanescent; and second, the characteristic

surface wave⁵, which is always evanescent, and obeys the relationship

$$\omega^2 = C \omega_B k \quad (2)$$

where ω and k are the angular frequency of the wave and wavenumber, respectively, ω_B is the local Vaisala-Brunt frequency, and ω_{an} is the non-isothermal acoustic cutoff frequency, given in terms of the temperature gradient α as⁵

$$\omega_{an}^2 = \frac{\gamma^2 g^2}{4C^2} + \frac{\gamma g}{2T} \alpha$$

where γ (=1.4) is the ratio of specific heats and g (=3.76 m s⁻²) is gravitational acceleration.

The type of oscillation generating Martian terminator waves can be located by finding α from equations (1) and (2). For example, the waves at +40° latitude occur when $V_T = C$; thus, the wave parameters are given by $V_T = C = \omega/(k \cos \theta)$, where θ is the solar declination. If we assume $\theta = 0^\circ$, then $\alpha = 4.26 \text{ K km}^{-1}$ from equation (1) and 25.6 K km^{-1} from equation (2). The first of these is the only realistic value.

It is worth studying Revolution 17 of Mariner 9. Terminator waves were observed at -20° latitude, 20°W longitude. Temperature profiles obtained during this revolution⁷ indicate that at this latitude the terminator was supersonic from an altitude of 30 km to at least 50 km. The minimum temperature of the tropopause at 38 km is 160 K, and it rises immediately above the tropopause with a gradient of 4 K km⁻¹. These experimental results certainly agree very closely with this theory.

There remains, however, one unusual feature of the Martian terminator wave observations. The tilt of the wavefronts corresponds to that expected during the Northern Hemispheric summer, whereas the latitudinal extent of the wave observations seems to indicate that the Northern Hemispheric stratosphere was at a lower temperature than the southern stratosphere.

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Effects of meridional winds on the interpretation of satellite inclination data

DURING the past decade, the analysis of long term changes in inclination of Earth-orbiting satellites has been used to determine characteristics of the zonal wind field in the Earth's upper atmosphere. As reviewed by King-Hele¹, results of analyses of this type indicate that, on average, the atmosphere between 200 and 350 km rotates faster than the Earth. Average eastward wind speeds of the order of 100 m s⁻¹ with respect to the Earth's surface are indicated in the altitude region around 300 km.

The effect of thermospheric winds on satellite inclination is quite small, of the order of 0.1° over a period of several months; as a result long and accurate observations are required to produce useful information. The deduced wind speed applies to a

height slightly above perigee and is most heavily weighted by the low latitude wind field, as the effect is strongest at the equator. Since at least several months of data are required for an adequate analysis, the inferred wind is an average over the local times and seasons covered by satellite perigee during that period.

Recent accurate satellite observations using Hewitt cameras have allowed shorter term changes in orbital inclination to be usefully studied. In this way, local time variations of upper atmospheric winds have been determined. Using data of this type for the orbit of Cosmos 316, King-Hele² tentatively inferred an 8-h oscillation in the zonal wind field at altitudes of 150–170 km. Here we show that such an interpretation of the data is not unique. In fact, we suggest that short term variations of the inclination may be more realistically explained in terms of meridional wind effects.

The zonal component of the upper atmospheric wind field is not the only component which affects satellite inclination. The meridional wind field can also have a notable effect, especially if its magnitude is as large as that of the zonal wind. King-Hele³ treated the effect of a meridional wind on satellite orbits and derived the following approximate relationship between the inclination i and the orbital period in days T_0 :

$$\frac{di}{dT_0} = \frac{1}{3\sqrt{F}} \left[\Lambda \sin i \left\{ (1-4e) \cos^2 \omega - \frac{1}{z} \cos 2\omega \right\} - \varepsilon (1-\kappa)^{1/2} \left\{ \left(1 - \frac{1}{4}\kappa\right) \left(1 - \frac{1}{2z}\right) \cos \omega - \frac{1}{4}\kappa \cos 3\omega \right\} \right], \quad (1)$$

where $\sqrt{F} = 1 - \Lambda T_0 \cos i$; Λ = atmospheric eastward angular velocity in units of the Earth rotation rate; e = orbital eccentricity; ω = argument of perigee; $z = ae/H$; a = semi-major axis of the orbit; H = atmospheric density scale height at perigee; ε = atmospheric northward angular velocity in units of Earth rotation rate; $\kappa = \sin^2 i / (2 - \sin^2 i)$.

This relationship is based on a number of reasonable approximations and is valid for $z \geq 5$. As pointed out by King-Hele, the zonal velocity (parameterised by Λ) leads to a secular change in i as the value of ω of the satellite orbit changes. The meridional velocity (parameterised by ε) has no net effect if ε is a constant and if the satellite orbit makes a complete cycle in ω . The first condition is probably not valid in the thermosphere, however, and the second is not satisfied when short term changes in inclination are studied.

Recent observations of the meridional wind field using the ionospheric radar backscatter technique (see, for example, Evans⁴) indicate that meridional winds can attain speeds of the order of 100 m s^{-1} and that there is a strong dependence on local time and season. Theoretical studies of thermospheric dynamics⁵ show an additional dependence on latitude. Since perigee latitude and local time and the season vary over the span of orbital data used in inclination analyses, so does the meridional wind ε . Thus, it seems probable that a non-negligible contribution to the change in a satellite's orbital inclination could result from interaction with the meridional wind field whether or not the satellite perigee completes a cycle in ω .

Perhaps even more relevant to the study of short term inclination changes is the observation that meridional wind effects generally will not average out when ω goes through only a fraction of a cycle. Although this is immediately evident from equation (1), we have, for purposes of illustration, integrated this equation for a nominal satellite orbit using various combinations of constant values of ε and Λ . The orbit used had the following initial values: $a = 7,682 \text{ km}$, $e = 0.15$, $i = 50^\circ$, $\omega = 100^\circ$. The initial perigee height for this orbit was 164 km and the parameter z was about 50. The atmospheric density was assumed to vary only with altitude (no latitudinal, seasonal or local time variation) and the vehicle drag parameter ($C_D A/W$) was taken to be $0.02 \text{ cm}^2 \text{ g}^{-1}$. The

time history of the orbit over a 5-month period was established using an orbit simulation computer program available at The Aerospace Corporation. It was found that the relevant orbital variables are described with accuracy sufficient for our purposes by the expressions:

$$\begin{aligned} \omega &= 100^\circ + 3t \\ e &= 0.15 - 0.00015t \\ T_0 &= (111.5 - 0.0286t)/1,440 \end{aligned}$$

where t is the time (d) from epoch.

Integration of equation (1) using the above expressions yields the results shown in Fig. 1 for four different combinations of Λ and ε : $\Lambda = 1.0$, $\varepsilon = 0$ (a); $\Lambda = 1.2$, $\varepsilon = 0$ (b); $\Lambda = 1.0$, $\varepsilon = 0.2$ (c); $\Lambda = 1.0$, $\varepsilon = 0.4$ (d). Curves *a* and *b* show the monotonic decrease in inclination that results from purely zonal winds, the larger value of Λ leading to a more rapid decrease in i . The slower variation of i near $T = 110 \text{ min}$ and $T = 108 \text{ min}$ occurs when the satellite perigee goes through apex in the Southern and Northern Hemispheres, respectively. Curves *c* and *d* show how meridional winds can affect the time change in inclination. After a complete cycle in ω (at $T = 108 \text{ min}$) the net effect of these constant meridional wind fields is seen to cancel out; but over a time period less (or more) than a com-

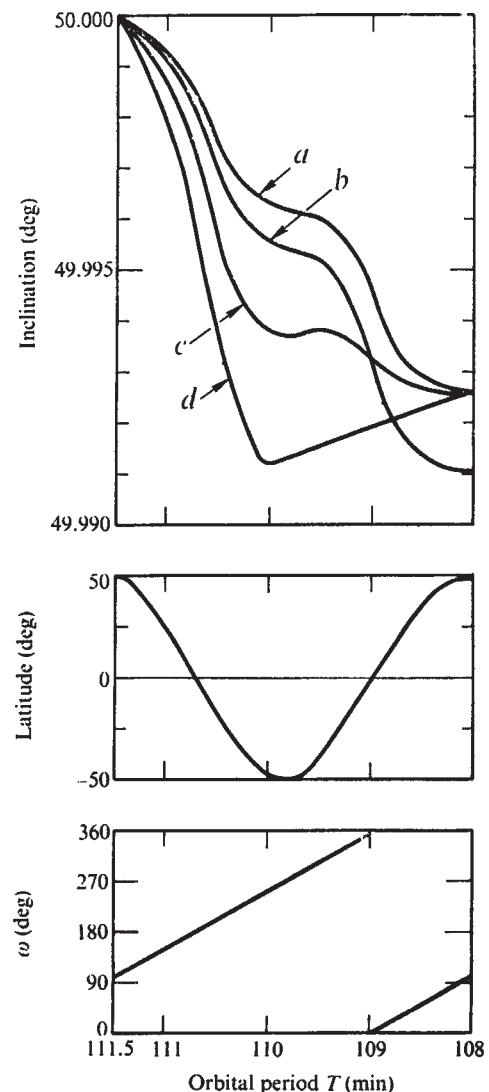


Fig. 1 Orbital inclination, latitude of perigee, and argument of perigee as functions of orbital period. The zonal and meridional wind parameters are: $\Lambda = 1.0$, $\varepsilon = 0$ (a); $\Lambda = 1.2$, $\varepsilon = 0$ (b); $\Lambda = 1.0$, $\varepsilon = 0.2$ (c); $\Lambda = 1.0$, $\varepsilon = 0.4$ (d).

plete cycle, the change in inclination is substantially affected by the meridional component. The rate of decrease in inclination, attributable to the prevailing zonal component, is modified by the meridional component to be faster or slower depending on whether the satellite motion is against or with the meridional wind. Thus, for example, if the earlier portions (up to $T=110$ min) of curves c or d were used to deduce zonal winds assuming no meridional wind effects, the inferred value of Λ would be larger than actually so. It is seen in curve d that if ε is large enough, the inclination can actually increase when the satellite moves in the same direction as the meridional wind. In fact, the inclination data for Cosmos 316 presented by King-Hele² show periods during which the inclination increases in a manner quite similar to that depicted in Fig. 1. In an analysis based on only zonal wind considerations, such data would be difficult to fit (as King-Hele acknowledged) and the best fit attainable would in essence disregard such features.

We emphasise that the curves presented here are only illustrative. As pointed out previously, the meridional wind field depends strongly on season, local time and latitude; therefore, in realistic situations the effect of meridional winds would be highly variable depending on the orientation of the satellite orbit in space. The meridional wind effects shown in Fig. 1 are undoubtedly extreme, as we have assumed ε to be constant and of magnitude comparable to peak values rather than zonally averaged values. Nevertheless, even with smaller values of ε the effect of meridional winds can easily modify the rate of change in inclination attributable to pure zonal winds. Thus, it seems that inclination data, particularly those encompassing less than a cycle in ω , cannot safely be interpreted on the basis of only zonal wind behaviour.

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Relativistic angular momentum of power circulation

THE purpose of this letter is to show that a special relativistic turning effect results from the angular momentum of the mass circulation connected with closed circuit mechanical or electromagnetic power transmission.

As a result of the relativistic equivalence of energy and mass, the momentum density of a body or a volume section through which power is transmitted is a function of the power flowing through it. In terms of relativity, this follows from the symmetry of the energy-momentum tensor¹. If $\mathbf{P}$ is the power flow per surface unit and c the velocity of light, the relativistic momentum density of the power flow is

$$\mathbf{g} = \mathbf{P}/c^2 \quad (1)$$

The direction of $\mathbf{g}$ is the same as that of the power flow, and $\mathbf{P}$ can be attributed to any kind of energy transmission, such as mechanical, thermal or electromagnetic. In the latter case, $\mathbf{P}$

is identical with the Poynting vector $\mathbf{P} = \mathbf{E} \times \mathbf{H}$ ($\mathbf{E}$ = electric field strength, $\mathbf{H}$ = magnetic field strength). For example, the relativistic momentum of a rotating line shaft or of an electric cable of length l transmitting power N is

$$G = Nl/c^2 \quad (2)$$

In other words, a body transmitting power can incorporate a momentum without moving in the direction of the momentum.

Of particular interest is the case in which power (that is, mass) circulates on a closed path within a body or a space section. A simple zero-loss power circuit may consist of an electric and a mechanical section: a loss-free electric motor drives a loss-free generator by a line shaft and the electric power delivered by the generator is transmitted back to the electric motor by a loss-free electric cable, so that the power circulates continuously between the electric motor and the generator. Each section of such a power circuit contains a momentum given by equation (2).

A closed mechanical power circuit can be obtained by connecting the ends of a flexible transmission shaft such that it forms a closed ring with mean radius R and that a torque M is conserved in the whole shaft. If the shaft is in rotation about its circular axis with angular velocity ω , a power flow $N=M\omega$ (a mass flow $m=N/c^2$) circulates through it. Equation (2) then yields the angular momentum of this power circulation:

$$J_1 = 2\pi R^2 M\omega/c^2 \quad (3)$$

In other words, the annular shaft possesses an angular momentum about the axis perpendicular to its plane (a axis) without rotating about this axis. If the shaft rotates freely about the a axis with an angular velocity Ω , then the total angular momentum about the a axis is $J = J_1 + \theta_1\Omega$, where θ_1 is the moment of inertia of the annular shaft about the a axis. Because of the law of conservation of angular momentum, a decrease of J_1 on account of a decreasing power circulation results in a corresponding increase of $\theta_1\Omega$. Suppose that ω slows down at a rate $d\omega/dt$ because of internal friction of the flexible shaft. Then the relation between the angular acceleration $d\Omega/dt$ about the a axis and the rate of decrease of power circulation $M d\omega/dt$ is

$$\begin{aligned} d\Omega/dt &= -(1/\theta_1) dJ_1/dt_1 \\ &= (-2\pi R^2 M/\theta_1 c^2) d\omega/dt \end{aligned} \quad (4)$$

Electromagnetic power circulation can easily be achieved in a closed superconducting circuit. Figure 1 resembles such a circuit and consists of a toroidal capacitor with a potential difference U between the inner and the outer toroid. Through the

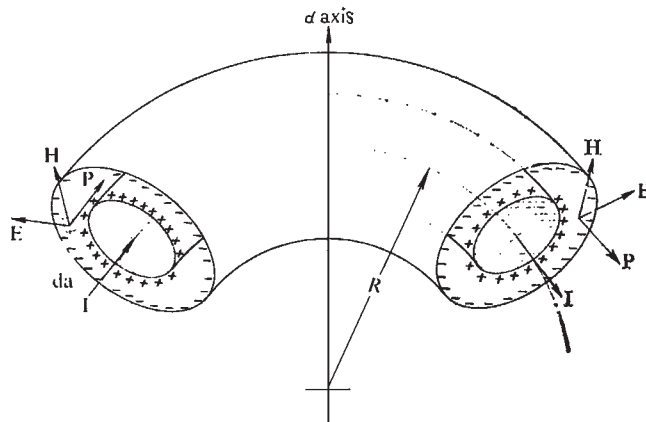


Fig. 1 Illustration of the power circulation in a toroidal capacitor. $\mathbf{P} = \mathbf{E} \times \mathbf{H}$ = Poynting vector; $\mathbf{E}$ = electric field strength; $\mathbf{H}$ = magnetic field strength; I = current through the inner superconducting toroid; da = area element of the cross section.

inner superconducting toroid a current I is flowing. At each point of the free space between the toroids there exists a magnetic field $\mathbf{H}$ and an electric field $\mathbf{E}$. The electromagnetic power flow within the toroids is given by

$$N = \int_1^2 \mathbf{P} \cdot d\mathbf{a} = UI \quad (5)$$

where $\mathbf{P}$ is the Poynting vector and $d\mathbf{a}$ is an area element of the cross section 1–2 perpendicular to $\mathbf{P}$. Equation (1) yields the momentum density of the electromagnetic field, and the angular momentum of the electromagnetic power circulation about the principal axis of the toroids (a axis) is given by

$$J_2 = (2\pi R^2 UI)/c^2 \quad (6)$$

where R is the mean radius of the toroids.

As the total angular momentum of the toroidal capacitor must be conserved, a change of the electromagnetic power circulation results in a transfer of angular momentum from the electromagnetic field, equation (6), to the conductors. Suppose that the electromagnetic power circulation changes at the rate $dN/dt = U dI/dt + I dU/dt$, then the corresponding angular acceleration of the toroids about the a axis is

$$\frac{d\Omega}{dt} = \frac{2\pi R^2}{\theta_2} \left[\frac{I}{c^2} \frac{dU}{dt} + \left(\frac{U}{c^2} - \frac{m_e}{e} \right) \frac{dI}{dt} \right] \quad (7)$$

with e/m_e the specific charge of the conduction electrons and θ_2 the moment of inertia of the toroids about the a axis.

A change of the electromagnetic power circulation can most easily be realised either by a change in the potential difference or by a change in the current intensity. In a practical laboratory experiment, a reduction of the potential difference U can easily be obtained by an internal discharge of the toroids; alternatively the current I can be reduced by heating the superconducting toroid above its critical temperature. Superconducting cables at present under development are designed for power capacities of up to 10 GW (equivalent to mass flow rates of 0.1 mg s^{-1}). Under laboratory conditions, much larger power capacities are possible^{2,3}. Suppose that a power of 10 GW circulates within a closed circuit cable with $R = 3 \text{ m}$. Equation (6) yields a relativistic angular momentum of $64 \text{ g cm}^2 \text{ s}^{-1}$, which should be measurable by present means.

The most intense power circulation achieved so far is in high power ring laser (such as the CO_2 laser at Los Alamos⁴). For example, a laser pulse of 10^4 J circulating in a path with $R = 3 \text{ m}$ has an angular momentum of $10^3 \text{ g cm}^2 \text{ s}^{-1}$.

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sedimentation or igneous and metamorphic activity. A gravity-folding model controlled by the structure of the pre-Cape rocks is proposed. De Swardt *et al.*² suggest that the Cape orogeny is of Alpine type, generally ascribed to gravity sliding away from a median zone of excessive uplift, with movement of the cover over a more rigid basement, which is accompanied by folding and thrusting. It is further pointed out that the best explanation for orogenies of Alpine type is that of collision of crustal plates⁴. Sharp³ implies that diamond-bearing kimberlites are the result of magmatic processes at the deep end of a subducted oceanic plate. The emplacement should occur deep beneath continents and the kimberlite intrusions parallel to fold mountain ranges. South African fields are stated as a specific example of the suggested process. Before the breakup of Gondwanaland, an oceanic plate was underriding the continent; the surface collision produced Cordilleran type mountains, the present Cape Fold ranges. It is shown that the diamond fields occur in bands parallel to the Cape Fold Belt. In spite of the fact that several prominent geophysical anomalies exist in the area, these models were exclusively based on geological arguments. We feel that useful reconstruction of the tectonic history must take the available geophysical evidence into consideration.

An array of three-component magnetometers recorded magnetic variation fields in 1971 over a large part of South Africa⁵. The principal result was the discovery of a linear body of highly-conductive material under the west central part of the Cape Fold Belt (Fig. 1). Although this structure passes south of the

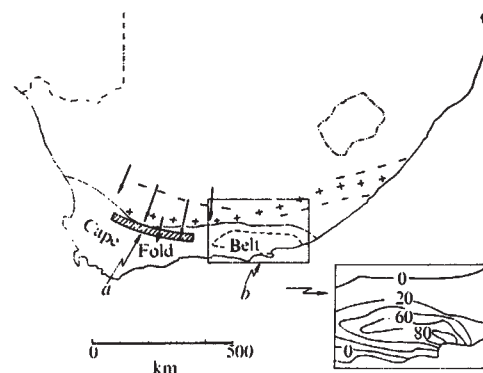


Fig. 1 Magnetic, gravity and conductivity anomalies in the southern point of Africa in relation to the Cape Fold Belt. The maximum (+) and minima (−) of the Beattie magnetic anomalies are indicated. The gravity anomalies are departures from an Airy model of isostatic compensation with crustal thickness 30 km. The inset map is adapted from ref. 6 and the gravity units are in mgal. The arrows represent the negative of the in-phase induction vectors from transfer functions calculated from the estimated normal horizontal components to the anomalous vertical variation field⁶. These vectors that point towards a conductive body are shown for a period of 128 min for the five stations nearest to the anomaly. The approximate position of the conductive structure is indicated. *a*, Conductivity anomaly; *b*, gravity anomaly.

southern corner of the triangular array it could be established that a large highly-conductive body elongated east–west underlies the Cape Fold Belt. Available information indicates that the conductor is in the lower crust or upper mantle. Further work is under way to define the width and axial line of the anomalous zone.

A large negative isostatic gravity anomaly exists in south-eastern South Africa at the eastern end of the Cape Fold Belt (Fig. 1). It reaches a minimum of -80 mgal of which about 20 mgal can be ascribed to Cretaceous sediments and is elongated along an east–west axis. This anomaly was originally explained as being caused by a relict root or thickening of the crust which had provided Airy-type compensation for a mountain range since eroded away^{6,7}. It was pointed out that a mountain range which would just be supported by the root

Plate tectonic origin for the Cape Fold Belt?

SEVERAL theories based on geological findings have been proposed for the origin of the Cape Fold Belt^{1–3}. An understanding of the tectonic conditions leading to the folding is not only of academic importance, but may be valuable in assessing the possibilities of finding oil in the southern Karroo.

Newton¹ argues that the Cape Fold Belt does not resemble a typical 'plate tectonics' orogen in respect of structural style,

would be 30 km wide and 1.4 km high. As large stresses are necessarily associated with isostatic anomalies of this large magnitude and area, a sequence of failures was predicted by Hales and Gough⁶, leading finally to uplift near the anomaly axis and reduction of the isostatic anomaly and the stresses. There are no large scale isostatic anomalies in the western half of the Cape Fold Belt which could be explained by postulating that the process of uplift is much more advanced there than in the east. Large vertical movement had in fact been observed in the western region⁸.

To explain the conductivity and gravity anomalies, Gough⁹ suggested that the isostatic anomaly might be the gravitational signature of an east-west trending linear plume in the upper mantle which also produces the conductivity anomaly under the west-central Cape Fold Belt.

The most prominent linear geophysical feature in South Africa is the Beattie Ridge magnetic anomaly¹⁰ which lies to the north of and, for most of its 900 km length, parallel to the Cape Fold Belt (Fig. 1). The anomaly consists of a maximum with amplitude ranging from about 100–500 ntesla (total intensity) flanked over large distances by long linear minima. No associated gravity anomaly exists. Deep geo-electrical soundings (J.H. de B., J.S.V. van Z., and F.K.B., unpublished) show that the body causing the anomaly is in the basement beneath the Karroo sediments. Model studies indicate that it is most likely in the middle or lower crust.

This magnetic anomaly cannot be explained in terms of the linear plume hypothesis. On the basis of the available geophysical evidence it is suggested that an oceanic plate underthrust Gondwanaland along one of its margins which coincided approximately with the present southern limit of Africa. Finally a continent–continent collision¹ occurred, producing mountain building which lasted from the Permian to middle Triassic¹¹. The most obvious result of this collision is the Cape Fold mountains of South Africa. Overfolding to the north is a prominent feature in these mountains, indicating that the pressure was directed mainly from the south¹¹.

Long linear magnetic anomalies are rarely observed on continents but are typical features in oceanic crust. The long linear Beattie magnetic anomalies may well be caused by the presence of large oceanic slabs thrust into the original continental edge during or after the formation of a subduction zone near the continental margin¹².

The roots of collision mountain belts are sialic¹ which explains the negative isostatic gravity anomaly associated with the Cape Fold Belt. The absence of an isostatic anomaly in the western half of the fold ranges can still be explained in terms of subsequent uplift.

Conductivity anomalies in the crust can be related to temperature¹³ or composition. Both types of conductivity anomalies are possible in the case of the Cape Fold Belt electromagnetic induction anomaly. If the conductivity anomaly is related to subduction of an oceanic plate and the eventual continental collision, it is most probably caused by a compositional effect. If it is connected to tectonic events surrounding the breakup of Gondwanaland and subsequent tectonic activity, however, there is a strong possibility that the anomaly is associated with anomalously high temperatures and partial melting and that the ridge is in fact a linear region of ascending mantle material as suggested by Gough⁹. This ambiguity as to the cause of the conductivity anomaly can be resolved by a study of the geo-thermal flux and seismic velocities in the area. This crucial information is not yet available.

Although some features remain unexplained, the geophysical evidence is consistent with the hypothesis that a continent–continent collision caused the formation of the Cape Fold Belt.

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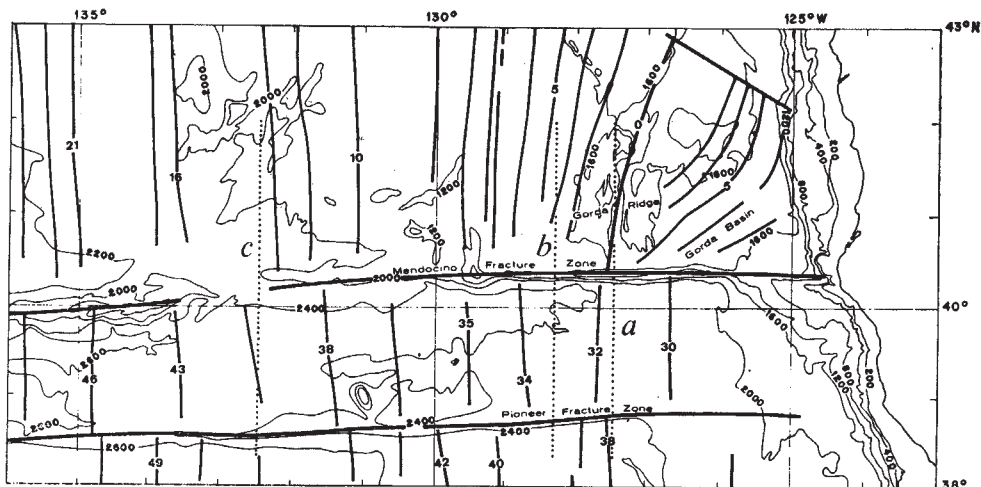
Thickness of lithosphere deduced from gravity edge effects across the Mendocino Fault

THE evolution of a lithospheric plate, as it migrates away from the accreting boundary (mid-ocean ridge crest), is mostly a result of vertical cooling by conduction. As density is a function of temperature and pressure, the density structure should be a function of the age of the plate and, in order to preserve isostatic equilibrium, the seafloor should subside as the plate cools¹. Thus, the variation of heat flow, seafloor depth and the gravity field are different expressions of the same process, progressive cooling, occurring over the whole thickness of the plate. Sclater and Francheteau² have verified these properties through an analysis of the variation of heat flow and depth with the age of the plate. Their model assumed that the plate remains a constant thickness and is floating in hydrostatic equilibrium over the asthenosphere. This led to an estimate of 75 km for the thickness of the plate.

Unfortunately, analysis of variation of the gravity field gives little information about structures in isostatic equilibrium with small lateral variations. Beyond the fact that the underlying volumes are of equal weight, the only information it gives comes from the influence of the lateral variation of density structure on the surface gravity field. As the evolution of the plate is slow, with a thermal constant of about 80 Myr (ref. 3), the lateral variations are small and difficult to detect, except perhaps near the accreting plate boundary⁴. Talwani *et al.*⁵ have shown, however, that over the Mid-Atlantic Ridge, gravity data combined with crustal seismic refraction data⁶ imply the existence of a low density upper mantle body of so-called 'anomalous mantle' at shallow depth (less than 40 km) below the surface. Their conclusion is in qualitative agreement with the low densities predicted by the plate tectonics model. But Morgan⁷ has argued that gravity data alone cannot rule out the existence of a much deeper compensating body. Keen and Tramontini⁸ have assumed that this compensation comes from a mass with a uniformly low density contrast, extending to depths of 200 km.

This controversy could be settled³ with gravity data alone, by studying active or fossil transform fault areas with large offsets, which juxtapose lithospheric plates of different ages (Fig. 1). The resulting large gravity edge effects are quite sensitive to the depths of the compensating masses. We show here that the gravity edge effect produced by the fossil Men-

Fig. 1 Bathymetric map, after Chase *et al.*¹⁸, showing gravity profiles *a*, *b*, and *c* (see text). Heavy lines, magnetic lineations with ages given in Myr (after Atwater and Menard¹¹). The Mendocino Fracture Zone juxtaposes sections of lithosphere which are of different ages: across profile *a*, 0.3 and 31 Myr; *b*, 3 and 33 Myr; *c*, 11 and 40 Myr.



docino Fault—a transform fault—can be explained with Sclater and Francheteau's plate model², and that the most probable thickness of the plate is 75 ± 25 km.

Figure 1 shows the location of three gravity profiles^{9,10} across the Mendocino Fracture Zone. Magnetic lineation data¹¹ show that the lithosphere on each side of the fault differs in age by 30 Myr. Figure 2 shows the free-air gravity anomaly profiles and the corresponding density models proposed^{9,10} to explain them. The crustal density structure adopted^{9,10} for profiles *a* and *b* (Fig. 2) are based on nearby seismic refraction measurements¹² using the Nafe and Drake velocity/density relationship¹³. There is no seismic refraction station near profile *c* (Fig. 1) but Dehlinger *et al.*^{9,10} projected results from refraction stations hundreds of kilometres away. In addition, they did not allow for the velocity anisotropy at the Moho interface¹⁴⁻¹⁶. The density structure they obtained for the mantle is, consequently, rather heterogeneous. In spite of these limitations, their results show the existence, on profiles *a* and *b* (Fig. 1), of an edge effect with a 70 mgal low about 100 km wide south of Mendocino. They explain this by invoking a compensating mass which is about 0.2 g cm^{-3} lighter in the upper part of the mantle, as originally proposed by Talwani *et al.*⁵. The edge effect decreases in amplitude but increases in width on profile *c* (Fig. 1), as predicted by the plate tectonics hypothesis of thermal evolution of the lithosphere.

We concentrate on profiles *a* and *b* (Fig. 1) which have a better crustal seismic refraction control and in which the contrast in thermal structure should be greatest, according to the plate tectonics model. The crustal density structure adopted was given by Dehlinger *et al.*^{9,10}. The density structure of the mantle is deduced from the vertical temperature distribution computed using Sclater and Francheteau's model² for a lithospheric plate of corresponding age. We also use their constants. The temperature of the asthenosphere is taken as $1,300^\circ \text{C}$ and the density of mantle material at 0°C as 3.4 g cm^{-3} . The only variable parameter is the thickness of the lithosphere, which we choose to vary between 25 and 200 km.

In practice, the density distribution within the lithosphere is computed by assuming that the ocean floor is the top of the lithosphere, but for the part of the lithosphere corresponding to the crust we adopt the density distribution deduced from seismic refraction results. We divide the remaining part of the lithosphere into layers of constant density differing by 0.01 g cm^{-3} ; the mean density of the layer is obtained from the Sclater and Francheteau model². Corresponding columns on each side of the fracture zone do not have exactly the same weight, because the temperature has been computed for a constant volume lithosphere and because the crust is ignored in the temperature-density model. We restore hydrostatic equilibrium between the two columns by applying to the older a small thickness correction which is proportional to the difference

between the mean computed density of a given layer and the density of the asthenosphere. We also ignore the modification of temperature structure which occurs because of heat flowing by conduction across the fossil transform fault. It can be shown that both simplifications introduce only second order modifica-

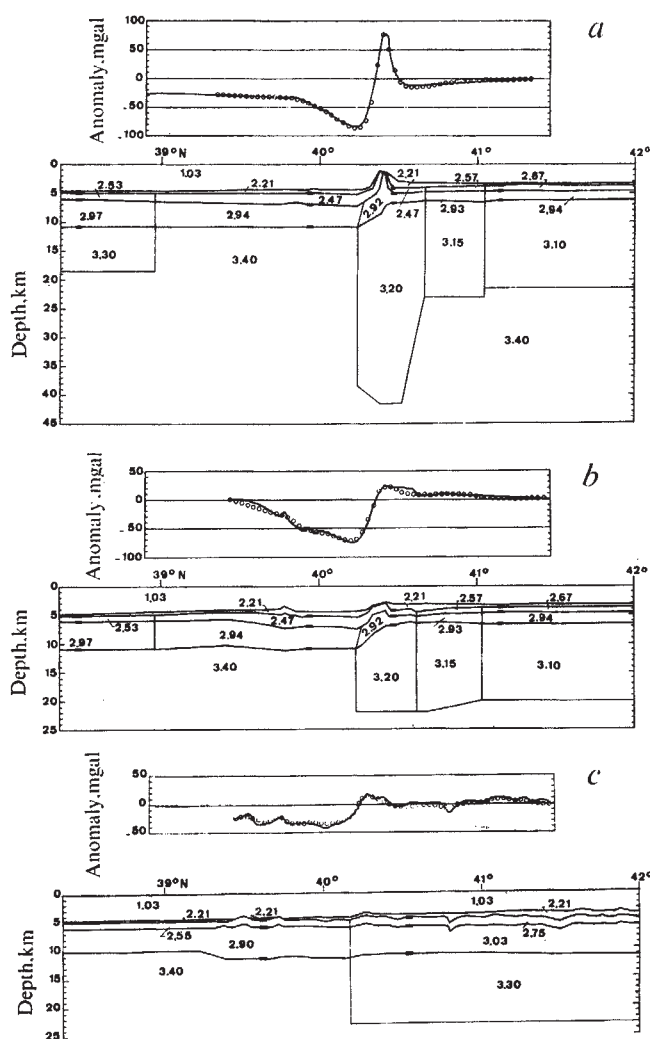


Fig. 2 Gravity profiles (free-air anomalies), and crustal sections along profiles *a* (along $127^\circ 20' \text{W}$), *b* (along $128^\circ 20' \text{W}$) and *c* (along $132^\circ 30' \text{W}$). In crustal sections, depth is shown from sea level, and densities (g cm^{-3}) are given. Data from refs 9 and 10.

tions. We felt that the many uncertainties in the data did not warrant testing more elaborate models, such as that proposed by Parker and Oldenburg¹⁷. Figure 3 shows the density models for profiles *a* and *b*.

Figure 3 shows the difference between the observed anomaly and computed gravity anomaly for different models in which the only parameter changed is the thickness of the lithosphere. If the plate tectonics model is correct and if the thickness of lithosphere adopted corresponds to reality, the resulting difference would be zero everywhere. If the thickness is too large then the edge effect is too large and the difference should be positive south of Mendocino and negative north of it. Comparison between computed and observed gravity anomalies shows that the best fits are obtained for a thickness of 75 km (Figs 3 and 4). The data are not good enough to exclude definitely a thickness as large as 100 km or as small as 50 km, but a thickness of 25 km is definitely too small and a thickness of 200 km too large. Thus, models of the type proposed by Morgan⁷ or Keen and Tramontini⁸ are not compatible with the data.

A thickness of 75 km also fits profile *c* where the crust is about 10 Myr older than it is near *a* and *b* (Fig. 4c). In the absence of nearby seismic refraction stations, we assumed that the crustal structures correspond to those given as a function of age by Goslin *et al.*¹⁸. In addition, the temperature structure may already be affected significantly by the conductive flow of heat through the fracture zone. Consequently, profile *c* is not as significant as the others.

The lithospheric thickness of 75 km obtained here is identical to that obtained by Sclater and Francheteau² on the basis of different types of data (heat flow and depth). This confirms that such a plate tectonics model is successful in accounting for the main physical characteristics of the evolution of a lithospheric plate in its first 40 Myr or so. It also considerably strengthens the choice of physical parameters. The thickness is actually an estimate for a plate 30–40 Myr old. The tempera-

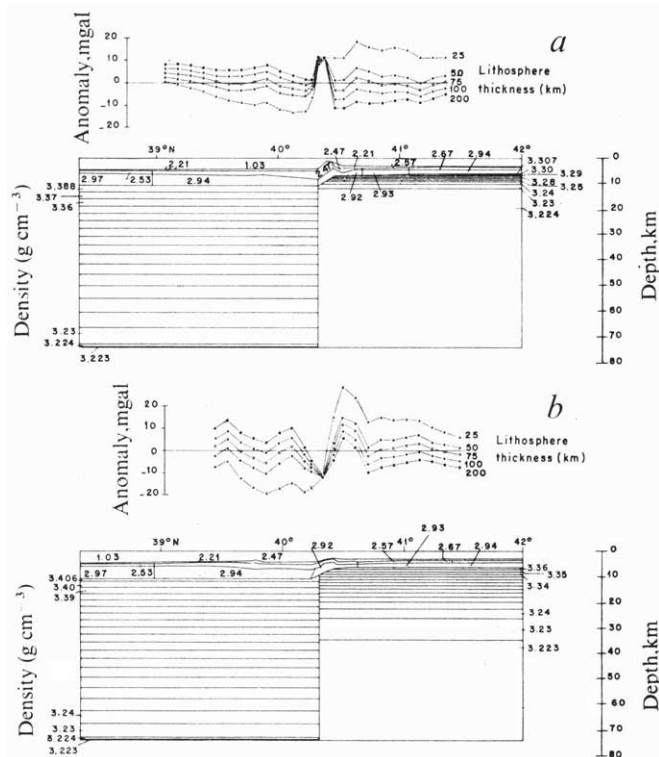


Fig. 3 Proposed density models across Mendocino Fracture Zone along profiles *a* and *b*. The thickness of the lithosphere chosen for the models is 75 km. The anomaly curves show the difference between observed and computed gravity anomalies for different lithosphere thicknesses. The density layers have been extended to infinity at both ends to avoid artificial edge effects.

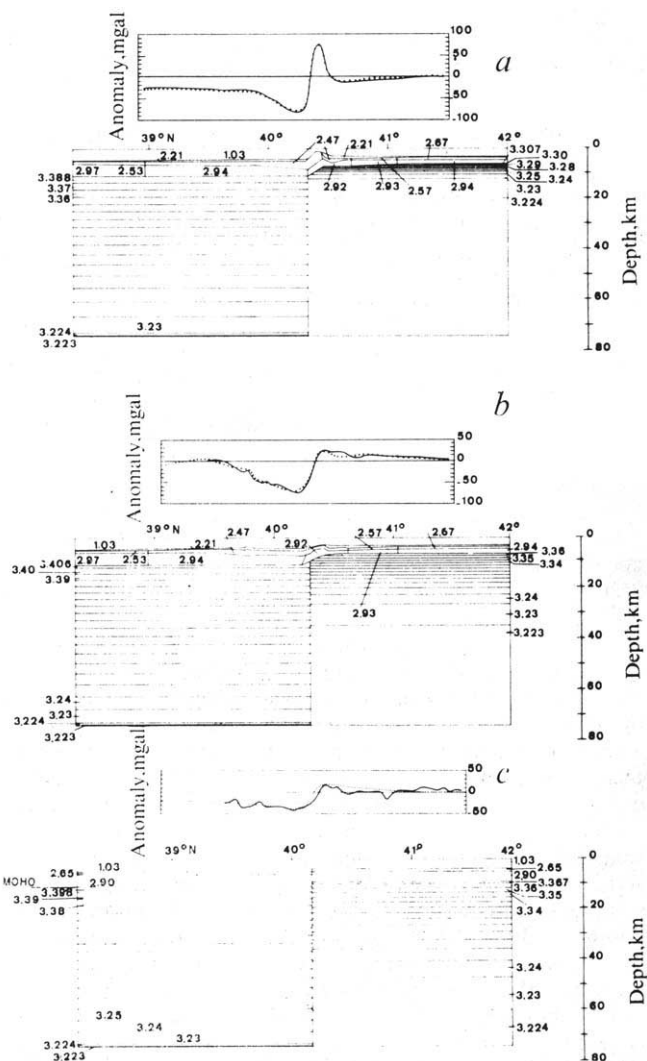


Fig. 4 Proposed density models across Mendocino Fracture Zone along profiles *a*, *b* and *c* and the corresponding observed (solid lines) and computed (dotted lines) gravity anomalies. The thicknesses of the lithosphere chosen for the models is 75 km. For *c*, the density model of the crust is a function of the age¹⁹ (see text).

ture structure at the accreting plate boundary does not depend on the thickness of the plate; that is controlled by the vertical convection term and not by the conduction term. Thus, this model can be compared with that proposed by Parker and Oldenburg¹⁷ in which the thickness, Z , of the lithosphere changes with age roughly according to the formula:

$$Z(t) = 9.4t^{1/2} \text{ km}$$

where t is time in Myr.

Thus, Z (30–40 Myr) $\sim$ 55 km and the estimate we obtain is in fair agreement with both models within the inherent uncertainties of the data and computations. We feel, however, that more systematic studies, with better seismic refraction and reflection control, across transform faults which juxtapose lithospheric plates of different ages should provide much better constraints to models of lithospheric evolution.

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Volcanism and glaciation during the past 40 millennia

CLOSE relationships between the timing of volcanic eruption and of glaciation in the past millennia have been noted¹⁻³ together with the possibility that the three main Neoglacial ice advances were contemporaneous with the three well dated volcanic activity periods in the Southern Andes³. Here, I show that glacial advance phases in the Western Northern, Eastern Northern and Southern Hemispheres are mainly synchronous with volcanic activity periods in New Zealand, Japan and southern South America over the past 40,000 yr (the period of reasonably accurate ¹⁴C measurements).

Information on ¹⁴C-dated volcanic eruptions and glacial advances is presented in Table 1 together with estimates of their relative magnitudes. All dates used in subsequent discussion are stated in ¹⁴C years. The volcanic eruption dates include all those summarised in refs 4, 5 and 6 for New Zealand, ref. 7 for Japan, ref. 1 (after Auer) for southern South America. The glacial advance dates were summarised from a global compilation of ¹⁴C-dated advances from many sources. These glacial dates were separated into phases of contiguous advance which were distinct from adjacent phases by at least 200 yr, with the exception of the glacial advance phases during the past two millennia which could be more finely separated. The estimates of magnitude in Table 1 were based on a scale of 1-3 as follows: for the volcanic ash dates, the magnitude of the New Zealand eruptions was determined from the published descriptions^{4,6} of the area covered by the

ash showers, with 3 = very widespread, 2 = widespread and fairly widespread and 1 = local. Estimates of the magnitude of Japanese volcanic eruptions were based on the descriptions accompanying the ¹⁴C-dated material⁷ as follows: 3 = a major eruption including eruptions which produced a thick pumice layer, 2 = a minor eruption, 1 = a mudflow or (rarely) a lava flow. For the Northern Hemisphere Pleistocene glacial advance phases, a value of 3 indicates a major mid-latitude continental ice advance, a value of 2 a moderate advance and a value of 1 a minor advance. For the subsequent 'Neoglacial' period which occurred after the last readvance of the Wisconsin ice sheet around 7,700-8,300 yr b.p., a value of 2 indicates a major advance of the alpine and polar glaciers and a value of 1 is a minor advance.

The dates for volcanic activity in Table 1 show a tendency for synchronous eruption phases in New Zealand, Japan and southern South America. All eight of the South American volcanic waves are identical with Japanese volcanic phases and six of these are also identical with New Zealand volcanic phases. Of the 18 New Zealand volcanic phases since 33,000 yr b.p., when Japanese dates are first available, 15 are synchronous with Japanese phases, 13 of these by variations of from 0 to less than 300 yr and the other two by variations of 400 and 600 yr. This tendency towards historical synchronicity can also be seen in volcanic eruption dates since 1500 AD (ref. 1) which show that major eruptions occurred in widely separated areas in 1660, 1693, 1694, 1783, 1831, 1846, 1886, 1888 and 1902 AD. Lamb¹ has stated that the existence of volcanic synchronicity seems to indicate that stresses build up within the Earth's crust which lead to simultaneous fractures over a wide region.

The dates for glacial advance in Table 1 outline the recognised periods of glacial activity in the late Wisconsin and Holocene. Major continental advances occurred ($\times 1,000$ ¹⁴C yr b.p.) around 41.0, 21.0-23.3, 19.4-19.7, 13.7-14.7 and 12.6-13.3, with moderate advances around 35.0-36.9, 30.5-33.5, 17.9-18.6, 16.5-17.2, 10.4-11.0 and 7.7-8.5. Following these large scale glacial advances, there were major Neoglacial advances of alpine and polar glaciers around 4.5-5.3, 2.1-2.9 and 0.04-0.5. The global synchronicity of glacial advance is again evident in the compiled dates in Table 1. The smaller number of Eastern Northern and Southern Hemisphere advance phases could indicate either insufficient knowledge or a genuine lack of glacial activity.

The proximity of the volcanic eruption and glacial advance dates in Table 1 is high. Because of limitations in laboratory technique, no ¹⁴C dates are available for Japanese volcanic eruptions before 33,200 yr b.p. and there are no dates before around 17,000 yr b.p. for the southern South American eruption waves. The only earlier information is the New Zealand dates of eruptions around $41,700 \pm 3,500$ and $41,000$ (67% probability) yr b.p. These dates correspond with the major Gahanna ice advance of the Eire lobe (Fig. 13 of ref. 8). There is no evidence of volcanism during the moderate glacial advance phase around 35,000-37,000 yr b.p. Following this advance phase, all subsequent glacial advances correspond fairly closely with dated volcanic eruptions except the minor advance phase around 15,600-15,700 yr b.p. for which no volcanic dates are available. In two instances there was a lag of 200-300 yr between the volcanic eruption and glacial advance dates: the very minor readvance around 9,200-9,500 yr b.p. which followed a volcanic phase around 9,500-9,800 yr b.p. and the last Wisconsin ice advance around 7,700-8,500 yr b.p. which followed volcanic eruptions around 8,650-8,950 yr b.p. These lags may be climatically significant if they indicate the time necessary for maximum ice advance to occur, once ice build-up has been triggered by volcanic action. Of the eighteen phases of volcanic activity and glacial advance since 17,200 yr b.p., all but four show a lag in ice advance of from 100 to 300 yr following initial volcanic eruption. In three other cases, there is no lag and in one instance, the date of glacial advance precedes volcanic action by 100 yr. Before 17,200 yr b.p. the volcanic and glacial phases are not as well defined; their ¹⁴C

Table 1 Periods of glacial advance and volcanic activity since 42,000 ^{14}C yr b.p.

Glacial advance dates ($\times 1,000$ ^{14}C yr b.p.)			Volcanic activity dates ($\times 1,000$ ^{14}C yr b.p.)				Southern South America	
Western Northern Hemisphere	Eastern Northern Hemisphere	Estimated magnitude	Southern Hemisphere	New Zealand	Estimated magnitude	Japan		Estimated magnitude
~41.0		3		41.0-41.7*	3			
35.0-36.0	36.0	2	36.2-36.9					
32.0	30.5-33.5	2		30.1*	2	30.2-33.2*	2,3	
27.0-28.0		1		26.3-27.9	-	26.3-27.9*	2	
24.6		1				24.1-24.9*	1	
21.7-23.3	21.0	3				21.4-23.0	3	
19.6	19.7	3	19.4	19.8-20.7	3	19.6-20.8*	3	
{ 17.9-18.5	18.0-18.4	2	18.6			17.9	1	
{ 17.0	17.2	2	16.5			16.5-17.3	2	16.0-17.0†
{ 15.6-15.7	15.6	1						
{ 13.7-14.7	13.9	3	14.1	13.8-14.7	2	13.9	1	13.5-14.0†
{ 12.6-13.3	13.2	3		12.5-13.4	2	12.7-13.1	3	
{ 11.5	11.8-11.9	1				12.0	1	11.9†
{ 10.4-11.0	10.5-10.7	2	10.6-11.0	11.2	2	10.4-10.6	1	10.9†
{ 9.2-9.3	9.2	1	9.5	9.5-9.8	1			
{ 7.7-8.3	7.8-7.9	2	8.5	8.6-8.8*	2	8.6	3	8.9
{ 6.5-6.8	6.4-7.2	2	6.8	7.3	2	6.5-7.2	1	
{ 5.7-6.1		1		6.2	-			
{ 4.7-5.3	4.5-5.0	2	4.5-5.3	5.1-5.2	1,2	4.8	2	4.7-5.4
{ 3.7	3.8	1	3.7			3.8	1	
{ 3.3	3.1-3.3	1		3.4	2	3.4	2	
{ 2.6-2.9	2.6-2.8	2	2.8-2.9	2.8	1,2	2.8	2	
{ 2.2-2.4	2.2-2.4	2	2.1-2.5	2.1-2.5	2	2.4-2.5	3	2.1-2.5
{ 1.5-1.8		1	1.5-1.6	1.8-1.9	2,3			
{ 1.0-1.4		1	1.0-1.3	1.2-1.4	2	1.3	2	
{ 0.6-0.8	0.7	1	0.7-0.8	0.8-0.9	1,2	0.7-0.9	1	
{ 0.04-0.5	0.04-0.45	2	0.04-0.5	0.06-0.47	1,2	0.062-0.31	2	0.05-0.45

* Standard deviation > 1,000 yr.

† Indirectly based on ^{14}C measurements.

accuracy is less and there is no consistent pattern of volcanic activity directly preceding glacial advance.

The relative magnitudes of the glacial and volcanic phases show a fairly close correspondence. The major glacial advance around 41,000 yr b.p. was contemporaneous with fairly to very widespread volcanic eruptions in New Zealand. The next major glacial advances occurred around 21,000-23,300 yr b.p. and around 19,400-19,700 yr b.p. Both these glacial phases were contemporaneous with periods of major volcanic eruptions in Japan, and the 19,400-19,700 yr b.p. ice advances occurred at the same time as a very widespread eruption in New Zealand. The last extensive ice advances of the Wisconsin age occurred during the Cary (Earliest Dryas) around 13,700-14,700 yr b.p. and the Port Huron (Older Dryas) advances around 12,600-13,300 yr b.p. The 13,700-14,700 yr b.p. advance phase occurred during moderate to minor volcanic phases in New Zealand and Japan and a major southern South American volcanic wave. The 12,600-13,300 yr b.p. glacial phase exactly corresponded to a period of moderate to major volcanism in New Zealand and Japan. During the Neoglacial, the three major advance phases of alpine and polar glaciers around 4,590-5,260, 2,100-2,940 and 40-460 yr b.p. were exactly contemporaneous with the three major post-Wisconsin volcanic activity phases around 4,700-5,450, 2,150-2,850 and 50-470 yr b.p. in New Zealand, Japan and southern South America as originally noted for the South American data³.

The temporal synchronicity of the glacial advance and volcanic activity phases shown in Table 1 may be more than coincidental, if the strong connection between volcanic ash and subsequent glacial advance demonstrated³ since 1500 AD reflects a valid causal relationship. The fact that both the late Wisconsin and Neoglacial ice advances are contemporaneous with major phases of volcanic activity suggests a volcanic trigger for glacial advance. Once this triggering occurs, the subsequent magnitude of glacial advance may depend partly on the magnitude of the volcanic activity and perhaps partly on autocatalytic mechanisms similar to those proposed by

Adam⁹ or Segota¹⁰, with the extent of glaciation related to position in the autocatalytic cycle. There is also a possibility that recurrent intervals of diminished solar activity may have created periods of cooler climate² which, if coincident with a volcanic activity phase, would have increased the effectiveness of the volcanic ash in stimulating glacial advance.

The close correspondence between periods of volcanic activity and glacial advance since 41,000 yr b.p., shown in Table 1 may have extended throughout the Quaternary. Fuchs and Paterson¹¹ have reported a similarity between the main epochs of Quaternary glaciation and volcanic activity in a number of regions of low latitude. Hamilton and Seliga¹² found that during the past 100 millennia the temperatures over the Antarctic and Greenland ice sheets were inversely proportional to the amount of volcanic dust which had fallen on these ice sheets.

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New dense phases of geophysical significance

THE major increases in density occurring at depths of 400–1,000 km in the Earth's mantle require the formation of phases in which silicon is six-coordinate. Such coordination has been demonstrated in stishovite (SiO_2 of rutile type¹), in the hollandite form of feldspar compositions², in the garnet form of $(\text{Mg,Fe})\text{SiO}_3$ ³, in garnet-type MnSiO_3 ⁴, in perovskite-type CaSiO_3 ⁵ and in SiP_2O_7 ⁶—but examples are still few. For the two compositional types believed to provide the bulk of the mantle, ASiO_3 and A_2SiO_4 where A is Mg or Fe, high pressure phase transformations of numerous model compounds such as MnGeO_3 , CaGeO_3 ⁶, Mn_2GeO_4 , and Ca_2GeO_4 ⁷ have demonstrated the possibility of high pressure ilmenite, perovskite, strontium plumbate and potassium nickel fluoride forms, respectively, as ultimate high pressure silicate phases; and disproportionation into simple oxides has also been discovered^{8,9}. In the absence of directly observed silicate transformations it is important to delineate the systematic crystal chemistry of each of these structural classes, particularly for those members containing ions closest in size to Fe, Mg and Si, and to obtain further examples of six-coordinate silicon in a range of compounds.

Since the ionic radius of Sc^{3+} (0.73 Å) is similar to those of Mg^{2+} and Fe^{2+} (0.72 and 0.77 Å) while that of Al^{3+} (0.54 Å) is comparable with that for octahedral Si^{4+} (0.40 Å), the composition ScAlO_3 seemed a suitable analogue for perovskite-type $(\text{Mg,Fe})\text{SiO}_3$, although it does not form a compound under normal conditions. We found that an equimolar mixture of amorphous Sc_2O_3 and Al_2O_3 (formed by heating the coprecipitated hydroxides in a vacuum), when reacted in an opposed anvil apparatus at 1,000°C and 120 kbar, gave an orthorhombic perovskite with $a = 4.933$, $b = 5.226$ and $c = 7.193$ Å, isostructural with the rare earth orthoferrites¹⁰.

Thus an ion of the size of Mg^{2+} can be accommodated in the A site of the perovskite lattice, while the high pressure behaviour of compositions CaTiO_3 – CaSiO_3 ³ have demonstrated that Si^{4+} can occupy the octahedral B site. It seems highly plausible that MgSiO_3 perovskite will become stable at very high pressures.

Structural refinement based on powder diffraction data showed Sc to be eight-coordinate with an average bond length of 2.25 Å, similar to that for Mg^{2+} in pyrope garnet¹¹. The Al—O_6 octahedron exhibits normal Al—O bond lengths. We would similarly expect that Si—O_6 octahedra in a perovskite form of MgSiO_3 would conform to the bond lengths found

Table 2 X-ray structure parameters for pyrochlore type $\text{Sc}_2\text{Si}_2\text{O}_7$ and $\text{In}_2\text{Si}_2\text{O}_7$

	$\text{Sc}_2\text{Si}_2\text{O}_7$	$\text{In}_2\text{Si}_2\text{O}_7$
a_0	9.287 ± 0.003 Å	9.413 ± 0.003 Å
No. of reflections refined	11	15
x	0.4300 ± 0.0012	0.428 ± 0.0016
$R = \sum(I_{\text{obs}} - I_{\text{calc}})/\sum I_{\text{obs}}$	0.0294	0.0235
$6 \times \text{Si—O}$	1.766 ± 0.007 Å*	1.794 ± 0.008 Å*

*90% confidence levels.

in rutile form SiO_2 , or in the pyrochlore silicates discussed below.

Consideration of the volume of the ideal perovskite cell as the product of its unit cell edges has led to a molar volume scheme in which the unit cell volumes of real perovskites are expressed as

$$V = C(A-O)^x(B-O)^{3-x}$$

where $(A-O)$ and $(B-O)$ are bond lengths taken from compounds of C-rare earth, rocksalt, rutile or corundum type. For more than 20 known $\text{A}^{2+}\text{B}^{3+}\text{O}_3$ perovskites, including high pressure phases, unit cell volumes can be fitted within $\pm 0.45\%$ by the values $C = 24.29$, $x = 0.970$, and extrapolation with considerable confidence made to the volumes of the geophysically important compositions MgSiO_3 , FeSiO_3 and CaSiO_3 and to solid solutions $(\text{Mg,Fe})\text{SiO}_3$ (Table 1). $\text{A}^{3+}\text{B}^{3+}\text{O}_3$ perovskite volumes can similarly be fitted using $C = 23.51$, $x = 1.159$. The densities predicted for MgSiO_3 and $(\text{Mg}_{0.86}\text{Fe}_{0.14})\text{SiO}_3$ are very close to those inferred for the zero-pressure densities of the shocked high pressure forms of these phases¹². Table 1 shows that $(\text{Mg,Fe})\text{SiO}_3$ perovskites would be 4–5% denser than isochemical oxide mixtures $(\text{Mg,Fe})\text{O} + \text{SiO}_2$ (stishovite). Consequently, even if disproportionation of MgSiO_3 – FeSiO_3 composition occurs in the transition zone^{8,9}, the oxides are likely to recombine in the deep mantle to form the denser perovskite phase.

In searching for additional examples of six-coordinate silicon we have found that thortveitite, $\text{Sc}_2\text{Si}_2\text{O}_7$, and its indium isotype, $\text{In}_2\text{Si}_2\text{O}_7$, are transformed at 1,000°C, 120 kbar to cubic pyrochlore type, with $a_0 = 9.287$ and 9.143 Å, respectively. In this $\text{A}_2\text{B}_2\text{O}_7$ structure, A ions are eight-coordinate and B ions six-coordinate. As the only variable atom position in the pyrochlore structure is $\text{O}(2)$, at $(1/8, 1/8, x)$, and all six Si—O bonds are equal, the silicate pyrochlores are particularly suited to structural refinement from powder data. The results are summarised in Table 2. An Si—O bond length in $\text{Sc}_2\text{Si}_2\text{O}_7$ of 1.766 ± 0.007 Å was obtained. The Si—O bond length in $\text{In}_2\text{Si}_2\text{O}_7$ is 1.794 ± 0.008 Å, a value different from that in the scandium compound. This effect may be due to the very marginal ionic radii fit of In and Si in the pyrochlore structure, although similar variations are observed in the polytypes of SiP_2O_7 ⁵. The average Si—O bond length for the two silicate pyrochlores (1.780 ± 0.014 Å) is, however, very close to that for rutile-type SiO_2 (1.775 ± 0.006 Å)¹³ and a value of 1.78 ± 0.01 Å would seem to define the values expected in high pressure mantle minerals.

Strong evidence has now been obtained (L. Liu, personal communication), by using laser heating of samples pressed between diamond anvils, for the existence of a perovskite phase close to MgSiO_3 in composition and with a unit cell volume almost identical to that predicted in Table 1.

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Table 1 Predicted* unit cell volumes ($Z = 4$) for silicates and other perovskites

Compound	Volume (Å ³) observed†	Volume (Å ³) calculated	Sum of formula volumes of constituent oxides‡ (Å ³)
BaPbO_3 ¹⁵	310.8	310.3	334.9
LaScO_3	266.1	264.1	—
YFeO_3	224.6	224.5	—
CaTiO_3	223.9	223.5	236.2
SrGeO_3 ¹⁶	218.9	220.5	248.1
CaGeO_3	206.4	206.0	222.0
MnVO_3	198.6	199.6	205.8
ScAlO_3	185.4	185.5	201.8
CaSiO_3	—	183.1	204.0
FeSiO_3	—	165.0	173.9
MgSiO_3	—	160.9	167.8
$(\text{Mg}_{0.86}\text{Fe}_{0.14})\text{SiO}_3$	—	161.5	168.7

* See text.

† From references cited in ref. 14 unless indicated otherwise in column 1.

‡ Rocksalt, rutile or corundum type.

§ Based on a unit cell volume (348 Å³) for Sc_2O_3 of hypothetical corundum form, by analogy with the C-rare earth and corundum volumes for In_2O_3 .

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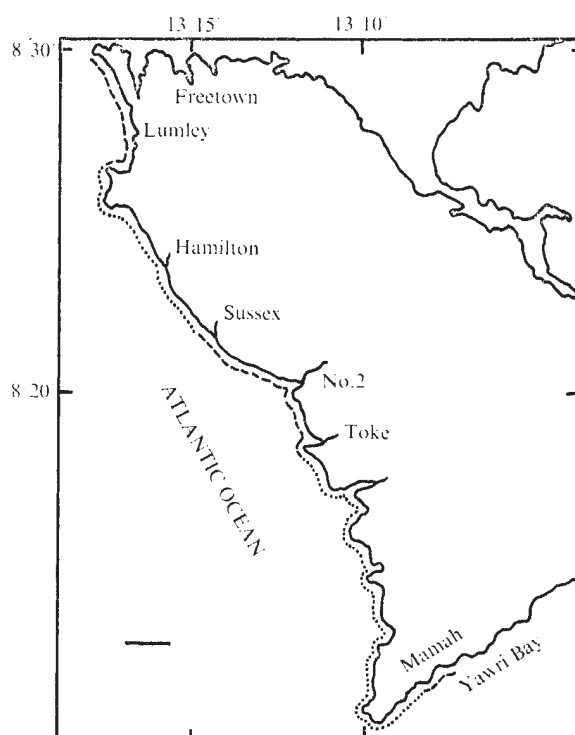


Fig. 1 Western area peninsula of Sierra Leone, showing the sites with tar polluted shores (dashed line) and the probable extent (dotted line) of the peninsular coastline affected by the pollutant. Scale bar represents 2.5 km.

Tar pollution of Sierra Leone beaches

THE widespread occurrence of pelagic tar and plastic wastes in parts of the Pacific and Atlantic oceans has been described previously¹. Extensive and considerable fouling of the sandy beaches of Sierra Leone by tar lumps has now been observed at Lumley, Sussex, No. 2, Toke and Mamah villages (Fig. 1) during the past 14 months (June, 1973 to July, 1974). Large quantities of soft, brownish-black lumps (up to 7–8 cm diameter) have been repeatedly washed ashore along the entire stretch (about 6 km) of Lumley beach. This reaches a maximum during June to August, probably because of onshore south-western Monsoonal winds and the increased eastward flow of the Guinea Current during May to October². On August 4, 1974, tar lumps were also observed 1–2 km up the No. 2 River estuary; and during Easter, 1974, a 4–5 km stretch of the sandy beach at Shenge village (7° 56'N 12° 56'W) at the southern tip of Yawri Bay, was also littered with the pollutant. Observations have not been made south of Shenge and north of Freetown (8° 29'N 13° 4'W) but the extent of current pollution tends to suggest that the whole Sierra Leone coastline, and perhaps those of the flanking countries as well, may be subject to frequent tar pollution, probably originating from the heavy, offshore traffic of tankers and ships.

At Lumley beach during ebb tide, tar lumps and other debris are deposited on the sand in a series of crescent-shaped aggregations by the receding waves. I have observed ghost crabs (*Ocypode* spp.) moving along these aggregations and from one crescent to another at different levels of the beach, probably as scavengers. Some workers¹ have already noted that the lumps are inhabited by a variety of marine organisms and I have seen several lumps (3–4 cm diameter) bearing colonies of 3–5 small goose barnacles. At succes-

sive spring tides, the lumps and the debris are swept by waves to the supralittoral fringe, leaving the littoral region momentarily clean until the next fresh influx of the pollutant. At the supralittoral fringe, tar lumps are lodged in the soft, dry sand and between the grass roots; here they harden, baked by the hot sun of the dry season (November to April). Fresh tar lumps easily stick to the feet, being effectively removed only by kerosene or local palm oil.

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In situ methylation of mercury in estuarine sediment

THE methylation of mercury compounds in sediments, believed to be a biological process, has been shown in a number of laboratory experiments^{1–5}. Background levels of methylmercury (CH_3Hg) have been reported for estuarine sediments from the Mississippi Delta, Mobile Bay and the Florida Everglades⁶. We now report that sediment in San Francisco Bay contained background levels of methylmercury and methylated mercuric chloride (HgCl_2) *in situ*.

Methylmercury was analysed using a Varian 1200 chromatograph equipped with an electron capture detector. The column (length 182.8 cm, internal diameter 0.318 cm) contained 5% 'butanediol succinate' on 'chromosorb W' 80–100 mesh and was maintained at 160° C. The presence of methylmercury was verified by comparing total mercury (Hg) with the amount of methylmercury in the extracts. This verification was carried out on an isotope shift Zeeman effect atomic absorption (IZAA) spectrometer⁷. Total mercury in sediment samples was also measured on the IZAA spectrometer. Methylmercury was extracted using a modification of the Westöo method⁸. Ten grams (wet weight) of sediment were placed in a 250 ml glass centrifuge bottle to which 25 ml H₂O, 10 ml concentrated HCl, 10 g NaCl, 2 ml 5% HgCl₂ (w/v) and 40 ml of benzene were added. The samples were shaken for 15 min and then centrifuged at 490 relative centrifugal force for 8 min.

Thirty millilitres of the benzene fraction were removed and placed in a 60 ml separating funnel. A 1% cysteine acetate solution (6 ml) was added and the samples shaken for 2 min. The cysteine acetate solution was withdrawn, measured and placed in a 30 ml separating funnel to which 3.6 ml 6 N HCl and 4 ml benzene were added. This solution was shaken for 2 min and allowed to stand for 10 min, the benzene was then removed and

Methylmercury was therefore produced *in situ* by San Francisco Bay sediments and natural background methylmercury was also observed. The amount of CH₃Hg produced *in situ* was far below that found under laboratory conditions¹⁻⁵ but significantly above background levels. The decrease in the amount of methylmercury found in most cases on day 28 could reflect a decrease in methylation resulting from a decrease in the amount of total mercury present by the diffusion of both methylmercury and inorganic mercury out of the experiment sediments. The loss of mercury was possibly aided by the degradation of methylmercury to its inorganic form, a phenomenon which has been shown to occur under laboratory conditions^{9,11-13}.

Andren and Harriss⁶ found that background levels of CH₃Hg decreased with sediment depth (sediment taken from Mobile Bay). This study has shown that at least in the initial phases of methylation, sediment at 6 cm produced more CH₃Hg than sediment at the surface.

The higher values of CH₃Hg in deep sediments could indicate: that more methylation occurs under anaerobic conditions; there is a greater loss of CH₃Hg from the shallower sediments; or there is a greater rate of CH₃Hg degradation in the shallow sediment.

Table 1 *In situ* methylmercury production (ng g⁻¹) by San Francisco Bay sediment

Sample	0	Day 18	28
10 p.p.m. Hg (shallow)	0.14	0.5	1.4
10 p.p.m. Hg (deep)	0.14	17.4	13.6
100 p.p.m. Hg (shallow)	0.14	28.3	0.0*
100 p.p.m. Hg (deep)	0.14	37.6	7.0
0 p.p.m. Hg (control)	0.14	0.1	0.0*

* Less than 0.01 ng g⁻¹.

stored for analysis. The extraction efficiency was 92.9% and the limit of detection was approximately 0.01 ng g⁻¹. Sediment was analysed for volatile solids⁹ and particle size distribution¹⁰.

To study methylation of HgCl₂ in the natural environment, wire mesh cylinders were embedded in the sediment of an intertidal zone of central San Francisco Bay. Sediment from the inside of each of the four cylinders was removed, separated and weighed; HgCl₂, dissolved in deionised water, was added to make 10 and 100 p.p.m. concentrations of Hg (wet weight) in each of two sediment zones (shallow and deep). Mercury was added to only one zone of each cylinder. The shallow zone was formed by replacing the appropriate mercury-sediment mixture only in the upper 1.27 cm of the cylinder. The deep zone was formed by replacing the bottom 6.35 cm with the mercury-sediment mixture and covering the upper zone with the original 1.27 cm of sediment. Duplicate 10 g sediment samples were taken from the cylinder on days 0, 18, and 28 of the experiment. Control samples were taken from areas adjacent to the experimental site.

The sediment which represented shallow and deep samples had an average volatile solids value of 2.5%. The particle size distribution was 55.0% medium and fine grained sand, 35.3% silt and 10.7% clay.

Methylation occurred in excess of background levels, being greatest in the deep zones. Background levels of methylmercury fluctuated over the study period from 0.01 to 0.14 p.p.b. CH₃Hg. We found that the fluctuations in total mercury as well as methylmercury concentration in San Francisco Bay sediment were a common occurrence throughout the year. Total mercury measurements during the study show a continuous decrease from the original values of 10 or 100 p.p.m. Hg. By the 28th day the initial 10 and 100 p.p.m. of Hg was reduced to 0.2 and 0.1 p.p.m., respectively, in the shallow sediment and 2.8 and 10.5 p.p.m., respectively, in the deep sediment. The *in situ* sediment had a total Hg content of 0.19 p.p.m.

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Upper mesospheric wind structure in Antarctica

UNDER a joint Indo-Soviet agreement I was the first Indian scientist to winter in Antarctica during the 17th Soviet Antarctic Expedition, 1971–73. In particular, I participated in the meteorological rocket soundings of the upper atmosphere carried out at Molodezhnaya, a station located at 67° 40'S, 45° 51'E at a height of 42 m above mean sealevel in Enderby Land, East Antarctica (Fig. 1).

In 1972 the mean annual temperature observed at the station was –10.5° C varying from a lowest minimum of –35.8° C to a highest maximum of 7.0° C. South-east winds prevailed at the surface with frequent gusts of 21–41 m s⁻¹. Annual mean relative humidity was 65% and the annual mean precipitation observed was 0.14 cm.

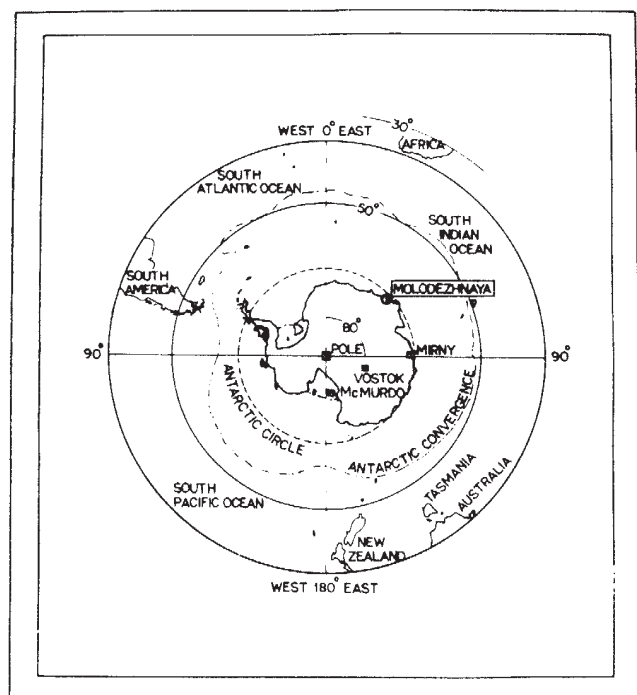


Fig. 1 Map of Antarctica and surrounding area showing the location of the station Molodezhnaya.

While I was in Antarctica, 60 M-100 meteorological rockets were launched from Molodezhnaya and 16 of these carried an additional wind sensor ('chaff') for determining the mesospheric winds. Most of the flights were successful and the average rocket apogee reached was 86.93 km. A summary of the chaff-borne flights, evenly distributed throughout 1972,

is given in Table 1. The results derived for the region 50–90 km (mostly for the 60–80 km layer in the upper mesosphere) are presented here. This is the first meteorological study of the upper mesospheric winds carried out in Antarctica.

The chaff used consisted of cylindrical aluminium-coated glass fibres having a diameter of about 0.025 mm. About 400 g of chaff, in a special container divided into two parts, was ejected at rocket apogee. The descending chaff cloud was then tracked by a high sensitivity Meteor-2 radar. Table 1 gives the corresponding wind tracks of all such flights. At the time of ejection at rocket apogee, above 80 km, the chaff fell at a high speed, about 160 m s⁻¹, but as it descended further it slowed down to about 4 m s⁻¹ at around 56 km because of the greater density and viscosity of the air at lower altitudes.

The radar data on the drift of the trajectory of the chaff are used to measure the wind speed and direction in the mesospheric region under study. Corrections for changes in the wind with altitude are also applied. The accuracy in measuring the wind speed at higher altitudes is about 6–10 m s⁻¹. Time-height cross sections for zonal and meridional winds are plotted using the conventional interpolation method in Figs 2 and 3.

The zonal components over Molodezhnaya, Antarctica, show that in the upper mesosphere the winds were predominantly easterly in the summer half and westerly in the winter half. In January and February (southern summer, Fig. 2) the speeds of the easterly winds ranged from 10 to 50 m s⁻¹ in the 60–80 km region for which the chaff data were available. On January 26, 1972, however, the zonal wind structure was found to have a small core of weak westerlies with wind speed about 10–15 m s⁻¹ in a narrow altitude region from 77 to 85 km. In February the easterly winds were stronger and attained a maximum speed of 96 m s⁻¹ at 82 km. During March there was only one rocket flight and during April there were none. During the first week of May, westerly winds of about 20 to 60 m s⁻¹ were detected. During the second week of May, however, the winds again became strong easterlies with a maximum speed of 113 m s⁻¹ at 66 km and a secondary maximum of 79 m s⁻¹ at 80 km.

Table 1 Summary of the 16 launchings of rockets containing a chaff sensor from Molodezhnaya, Antarctica, in 1972

Date	Time (GMT)	Rocket apogee (km)	Wind track (km)		60–70 km MsCl ₆₅ (m s ⁻¹)	65–75 km MsCl ₇₀ (m s ⁻¹)	70–80 km MsCl ₇₅ (m s ⁻¹)	75–85 km MsCl ₈₀ (m s ⁻¹)
January 5	1450	82.80	80–62	Meridional	N/A	6.1	2.4	N/A
				Zonal	N/A	–40.1	–41.5	N/A
January 19	1440	84.65	84–64	Meridional	N/A	3.3	5.5	–2.5
				Zonal	N/A	–38.2	–29.3	–16.4
January 26	1435	88.03	86–62	Meridional	N/A	–14.9	–16.4	–6.7
				Zonal	N/A	–33.3	–13.1	6.2
February 16	1535	83.70	82–60	Meridional	–3.4	–2.6	5.1	N/A
				Zonal	–21.2	–26.7	–30.5	N/A
March 1	0330	87.50	86–56	Meridional	9.3	9.3	14.5	12.8
				Zonal	–12.4	–26.3	–33.9	–46.0
May 3	1400	88.00	84–56	Meridional	1.9	–12.7	–24.2	6.4
				Zonal	29.0	11.3	2.3	–21.1
May 6	1410	91.50	90–58	Meridional	3.4	20.9	–3.9	–6.2
				Zonal	45.0	27.7	33.0	19.6
May 17	1750	87.94	84–56	Meridional	–31.1	–17.8	–50.1	–44.0
				Zonal	–61.5	–57.0	–37.5	–9.5
June 21	1400	92.85	88–56	Meridional	–5.8	–8.1	–18.2	–25.6
				Zonal	54.2	34.2	13.6	14.0
June 28	1405	88.40	84–54	Meridional	29.7	23.0	5.4	–6.4
				Zonal	89.2	60.6	16.8	17.2
July 5	1400	86.70	84–54	Meridional	12.1	17.9	14.7	16.2
				Zonal	116.6	74.0	32.5	16.3
July 19	1400	85.30	84–56	Meridional	10.1	0.4	–12.7	–0.9
				Zonal	32.4	21.7	38.9	41.3
September 6	1400	88.44	82–62	Meridional	–15.6	–28.0	0.2	N/A
				Zonal	36.2	0.9	10.4	N/A
September 20	1405	86.55	66–52	Meridional	4.3	N/A	N/A	N/A
				Zonal	50.9	N/A	N/A	N/A
October 4	1353	86.50	80–58	Meridional	6.7	4.3	3.1	N/A
				Zonal	12.8	4.7	–1.6	N/A
December 20	1430	81.98	80–64	Meridional	N/A	10.6	7.5	N/A
				Zonal	N/A	–36.3	–38.7	N/A

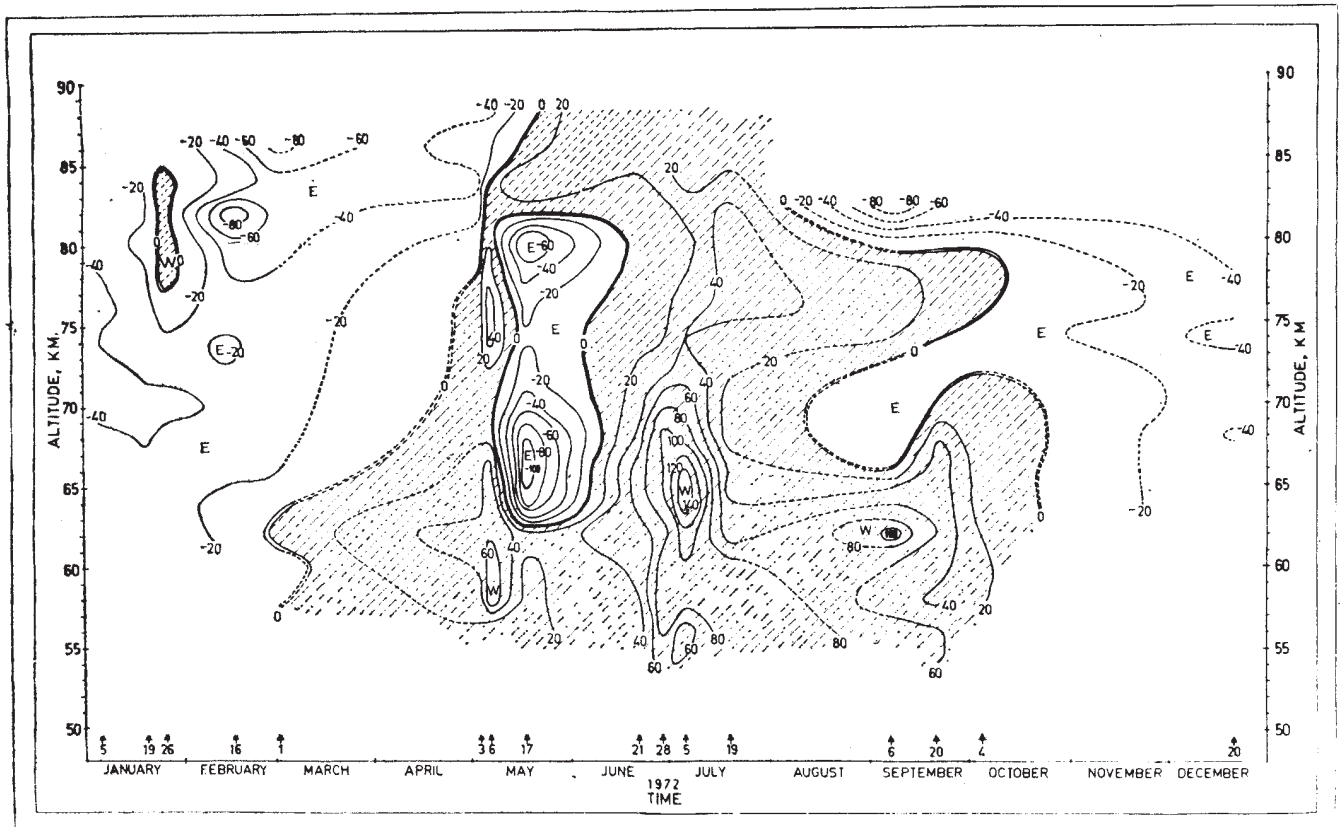


Fig. 2 Time-height cross section for Molodezhnaya zonal winds (1972) with west (+, shaded areas) and east (−) components in m s^{-1} . ---, Extrapolated data. Arrows above the abscissa show the date on which data were obtained.

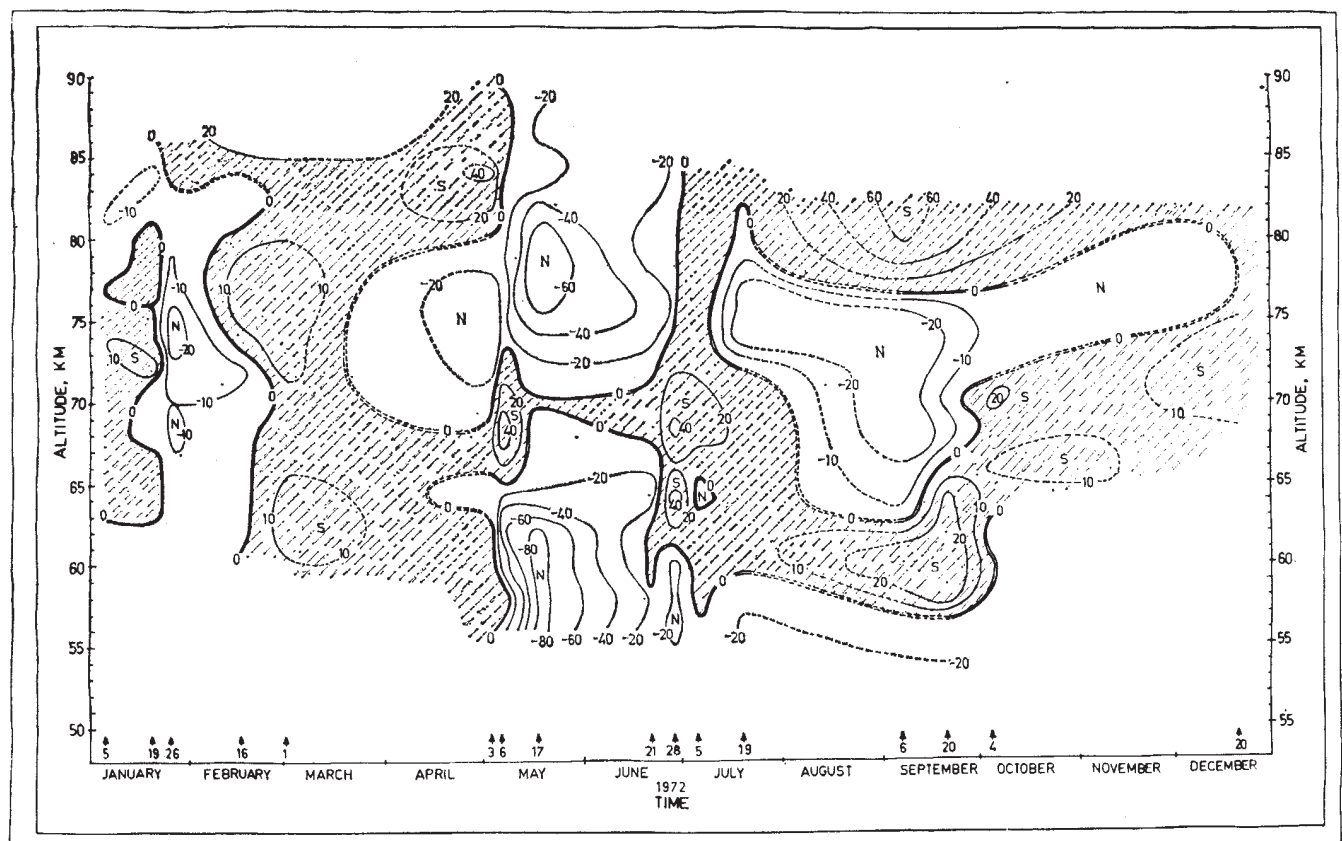


Fig. 3 Time-height cross section for Molodezhnaya meridional winds (1972) with south (+, shaded areas) and north (−) components in m s^{-1} . ---, Extrapolated data. Arrows above the abscissa show the date on which data were obtained.

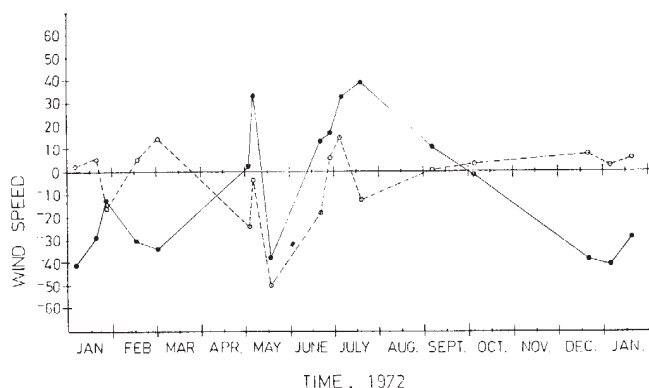


Fig. 4 Zonal flow (—) obtained by averaging winds from the individual soundings over a 10-km layer centred at 75 km (Mesospheric Circulation Index, MsCI). ---, Meridional flow. Positive values refer to west and south components. Wind speeds in m s^{-1} .

In June and July (southern winter) a westerly jet was formed which attained a maximum speed of 157 m s^{-1} at 64 km and became weaker at higher altitudes. The strong westerly winds started to weaken in September–October (southern spring) in the 60–70 km region and weak easterlies started developing above 70 km. There were no ascents with chaff payloads during August and November. In December the zonal flow was characterised by easterlies of about $20\text{--}40 \text{ m s}^{-1}$.

The meridional cross section of the winds shows that they were of variable nature (Fig. 3). In January weak southerly winds of about $5\text{--}15 \text{ m s}^{-1}$ predominated in the upper mesosphere. In February the winds were mainly northerlies, ranging from $5\text{--}25 \text{ m s}^{-1}$. In May the northerly winds became stronger and formed a jet which had a maximum speed of 88 m s^{-1} at around 60 km, with a secondary maximum of 79 m s^{-1} at 78 km. In the narrow region 65–70 km there was a small core of southerlies having a maximum speed of 57 m s^{-1} at 68 km.

In June and July the northerly winds changed to southerly ones with a maximum speed of 48 m s^{-1} at 64 km. In September and October the winds were mainly southerlies of about $10\text{--}60 \text{ m s}^{-1}$ with lower values at around 60 km and higher values at around 82 km. There was a core of weak northerlies at around 75 km. In December the meridional flow was characterised by weak southerlies of about $5\text{--}15 \text{ m s}^{-1}$.

The seasonal reversal of winds at different times of the year can be shown clearly by a method devised by Webb¹. In analysing the Meteorological Rocket Network (MRN) data Webb¹ calculated an average flow over a layer 10 km thick centred at 50 km and called it the Stratospheric Circulation Index (SCI) of the appropriate layer. Adopting the same method, I have computed mean values of the wind speeds in m s^{-1} obtained from the individual soundings by averaging over a 10-km layer centred at each of the altitudes 65, 70, 75 and 80 km (Table 1). Since this presents an average flow of the mesosphere under study, it is termed the Mesospheric Circulation Index (MsCI). Its values for the 70–80 km layer centred at 75 km, MsCI_{75} , are plotted in Fig. 4.

In conclusion, I found that zonal winds were predominantly easterly in the summer half and westerly in the winter half whereas the meridional winds were variable. From the analysis of the MsCI (Fig. 4) it is obvious that the summer easterly flow changed to a westerly flow in the first week of May, followed by a rapid reversal to an easterly flow in the second week. The easterly flow again changed to westerly flow in mid June. The meridional flow (Fig. 4) also showed a rapid shift

from northerlies to southerlies in the last week of June. The southerly flow again rapidly changed to northerly flow in mid July. These rapid shifts in both zonal and meridional components indicated a sudden 'explosive' change in temperature distribution which might have occurred in the upper mesosphere. I am at present investigating this phenomenon. The winter westerly flow changed to the summer easterly flow at about the end of September. It seems that both for the zonal and the meridional flows the summer–winter shift was a rapid and dramatic change, whereas the winter–summer shift was slow and unspectacular. I am also studying diffusion phenomena in the mesosphere using the chaff data.

I acknowledge the collaboration of the members of the 17th Soviet Antarctic Expedition, 1971–73, and thank the Hydrometeorological Service of the USSR and the Department of Atomic Energy, Government of India, for allowing me to take part in the expedition, and giving me the privilege of being the first Indian Explorer of Antarctica, which was made possible by the late Professor Vikram A. Sarabhai. I also thank Professor P. R. Pisharoty for stimulating guidance and encouragement.

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Flow near an oscillating cylinder in dilute viscoelastic fluid

THERE are many natural phenomena in which nonlinear interactions of time-dependent inputs give rise to steady—that is, time-independent outputs. One of these is a steady streaming belonging to a class of secondary flows sometimes called acoustic streaming. It occurs when a circular cylinder oscillates normal to its axis in an unbounded Newtonian fluid^{1–3}. We report here on the steady secondary flow induced when a long thin cylinder oscillates as described in a viscoelastic liquid. We found that the direction of steady streaming is opposite to that found for the bulk of fluid when the experiment is performed with a Newtonian fluid.

Reversal of secondary flow has been noted in several flows^{4–6} when fluid has been altered from Newtonian to viscoelastic behaviour.

Our experimental apparatus (Fig. 1) was adapted from that used by Bertelson *et al.*³. The oscillating brass cylinder was 6.72 cm long, and had a diameter of 1.6 mm. It was suspended on two bronze leaf springs, each 0.15 mm thick. The oscillating system was contained in a cylindrical glass container 9.55 cm long and 7.05 cm in diameter. The glass container was mounted in a strong, fixed magnetic field. Oscillation was induced by an alternating current (Fig. 1).

The container was filled with test fluid containing tracer particles (hollow glass spheres 40–60 μm in diameter). Pathlines representing steady secondary streaming were obtained by photographing particles in a plane perpendicular to the axis of the vibrating cylinder. Only the secondary flow appears in the photographs because illumination is provided by a stroboscopic source synchronised with the frequency of oscillation.

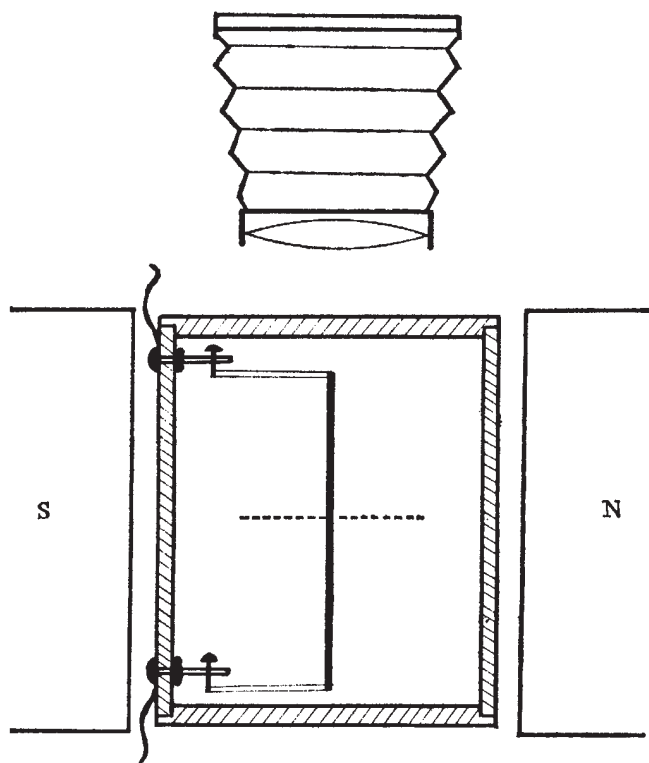
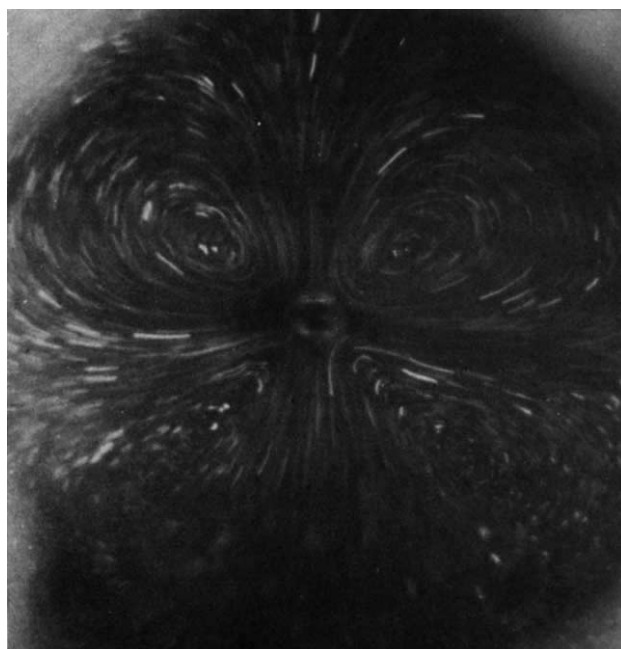


Fig. 1 Schematic diagram of apparatus. Dotted line indicates plane of illumination. S and N, Magnets.



Photographs were first taken with a Newtonian test fluid: a 50% by weight solution of glycerine in water. The viscosity of the liquid was approximately $6 \times 10^{-3} \text{ N s m}^{-2}$. The oscillating frequency was set at 45 Hz. For small amplitudes of oscillation (approximately 25% of the cylinder diameter) the results shown in Fig 2a were obtained. There are two regions of flow (see ref. 3). In the inner region near the oscillating cylinder the pathlines form small closed vortices with fluid flowing toward the oscillating cylinder in the direction of oscillation and away from it in the transverse direction (Fig. 2b). Of more predominance is the outer region which also contains closed vortices but with an opposite sense of rotation. Thus, the predominant secondary flow is away from the oscillating cylinder along the axis parallel to the direction of oscillation. (At lower Reynolds numbers a thicker inner vortex system can be obtained⁷.)

The contrast is striking when the results of Fig. 2 are compared with those of a dilute viscoelastic fluid possessing drag reducing properties⁸. The secondary flow shown in Fig. 3 was obtained under conditions similar to those cited for Fig. 2 with one exception. The glycerine-water solution contained 0.01% by weight of Dow Separan AP30, a water-soluble polyacrylamide. No small inner vortex is visible in Fig. 3, and the predominant secondary flow is toward the oscillating cylinder along the axis parallel to the direction of oscillation. These results could signify that the small inner vortex observed with the Newtonian fluid is greatly enlarged in the experiments performed with polymer solution.

In Figs 2a and 3a there is an asymmetry between upper and lower portions of the photographs. That is because the cylinder motion was not truly rectilinear, but followed an arc determined by the radius of the spring supports. The experiment of Fig. 3a was performed at a slightly higher amplitude causing more predominant curvature.

As the addition of 100 p.p.m. of Separan has little effect on the shear viscosity of the glycerine-water solution, the drastic change in secondary flow probably results from the elasticity of the Separan solution.

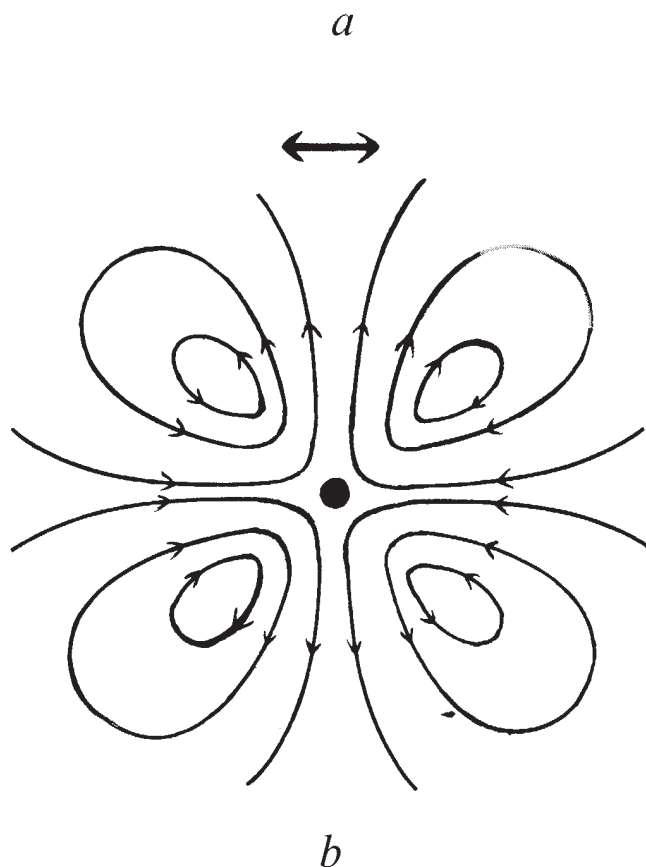
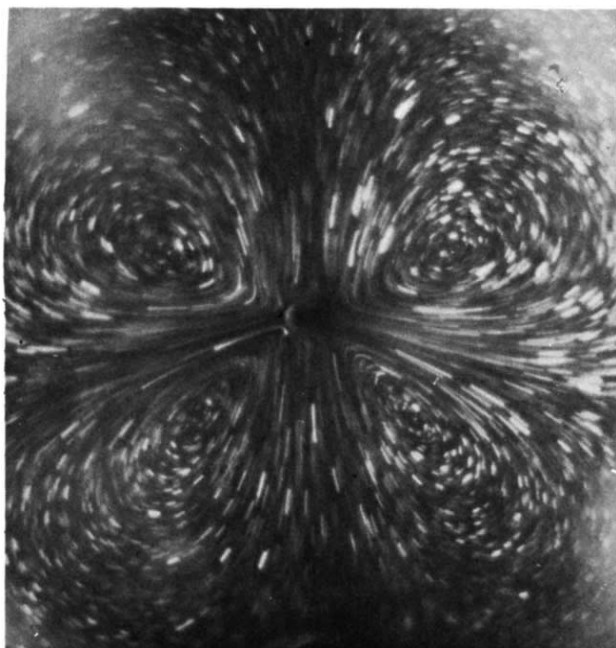
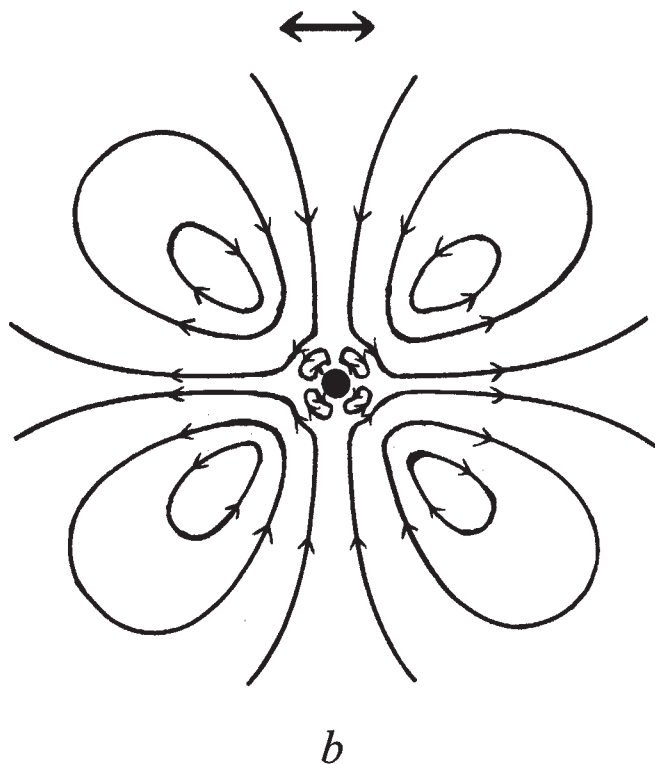


Fig. 2 Steady secondary flow for Newtonian fluid: 50% by weight glycerine in water. Frequency of oscillation, 45 Hz. a, Tracer photographs at $f/2.8$ and 1 s exposure time, showing inner and outer vortices; b, sketch drawn from a showing direction of circulation. Double arrow, direction of cylinder oscillation.



a



b

Fig. 3 Steady secondary flow for viscoelastic fluid: 50% by weight glycerine in water, with 0.01% by weight of Dow Separan AP30. *a*, Tracer photographs at $f/2.8$ and 1 s exposure time, showing absence of identifiable inner vortex analogous to Fig. 2; *b*, sketch drawn from *a* showing direction of circulation.

Further experiments with varying amounts of Separan showed that the reversal of flow persisted to very dilute solutions. Secondary flow characteristic of Newtonian flow only reappeared when the Separan was reduced to 10–20 p.p.m.

Hopefully, this new phenomenon may be helpful for characterisation and, perhaps, further elucidation of the nature of dilute solutions of drag reducing polymers.

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Taylor vortex instability and annulus length effects

THE stability of the flow between rotating cylinders has both fundamental and technological significance. The classical analysis by G. I. Taylor¹ of the stability of the circumferential laminar flow deals with coaxial cylinders for which the length L is infinite and the radial clearance $c = R_2 - R_1$, is small, but subsequent work² has eliminated the need for the small clearance approximation. The flow, once unstable, takes the form of axisymmetric toroidal vortices and these Taylor vortices can in turn become unstable with the onset of non-axisymmetric wavy vortices. Early analysis of this instability³ was again restricted to coaxial cylinders of infinite length and small clearance, but Eagles⁴ has produced a stability analysis avoiding the small clearance approximation and has applied it to a cylinder radius ratio $R_1/R_2 = 0.951$. No stability analyses seem to exist for an annulus of finite length and most experimental workers choose to work with long cylinders even though short cylinders are more likely to be encountered in engineering applications. Recently I described some observations⁵ of vortex sizes in short annular clearances and I noticed the apparent absence of wavy vortices in those experiments: now I have more positive evidence of the considerable effect of annulus length on the wavy vortex critical speed.

The present flow visualisation results are for a rotor of diameter 91.42 mm concentrically positioned inside a Perspex stator of bore 103.2 mm, thickness 19 mm and effective length 250 mm, so that $R_1/R_2 = 0.894$. Vortices in the test fluid, a silicone oil of kinematic viscosity $\nu = 19.3$ centistoke, were made visible by adding a small quantity of aluminium particles of typical maximum dimensions 0.01 mm. Given a high standard of control of rotor speed (N revolutions s^{-1}) and fluid temperature, this commonly-used visualisation technique enabled the critical speeds to be determined repeatedly to better than

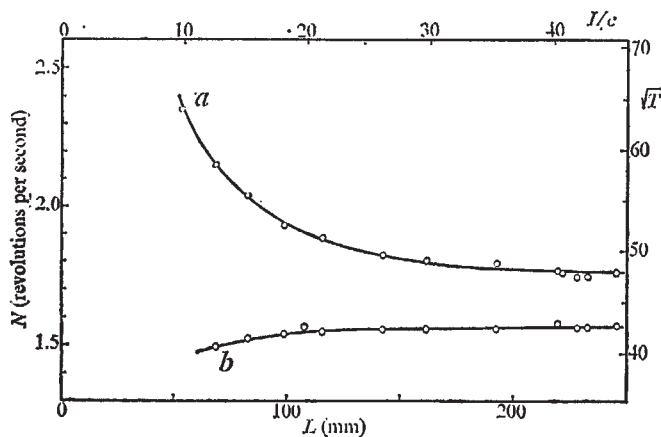


Fig. 1 Effect of annulus length L on critical speeds N determined from visual observations. a , Wavy vortices; b , Taylor vortices. $R_1/R_2=0.894$.

$\pm 0.5\%$ in general, with no hysteresis apparent for speed increasing and decreasing. Annulus length was varied by changing the depth of the test fluid, and the end boundary conditions were asymmetric, with the lower end a stationary solid surface and the upper a free liquid surface. Table 1 shows that observed critical speeds are in satisfactory agreement with calculated values for the maximum annulus length tested. Figure 1 shows that length has a small effect on the speed at which Taylor vortices appear but a marked effect on the speed at which wavy vortices appear: at $L/c=25$ the second critical speed is about 10% higher than the calculated value and at $L/c=10$ about 40% higher.

To achieve greater length: clearance ratios, observations were also made using a rotor of diameter 94.45 mm inside a stator of diameter 100.01 mm, giving a radius ratio $R_1/R_2=0.954$. The silicone oil viscosity was $\nu=6.10$ centistoke at the controlled operating temperature. Here, critical speeds were determined from measurements of the torque reaction on the stator which was supported on externally-pressurised air bearings: the onset of Taylor vortices produced an upward discontinuity in the slope of the torque-speed relationship as speed was increased in small steps and the onset of wavy vortices produced a later and less pronounced downward discontinuity. This method also gave good results, repeatable to better than $\pm 0.5\%$ at larger lengths but with slightly diminished accuracy at short lengths where the slope changes became somewhat rounded. For $L/c < 10$, the Taylor critical in particular was less certain as the torque-speed relationship became nonlinear and slightly concave upwards before the major change of slope occurred: the latter was taken as the critical

condition. The results, shown in Fig. 2, are generally similar to those in Fig. 1 although the very small variation in the Taylor critical speed with length is of opposite sign to that in the visualisation experiment. The critical speed for the onset of wavy vortices begins to rise appreciably as L/c values are reduced below 40 and the maximum value measured was about 22% above the minimum. Table 1 shows that observed critical speeds at the maximum L/c value of 107 are in satisfactory agreement with theoretical values.

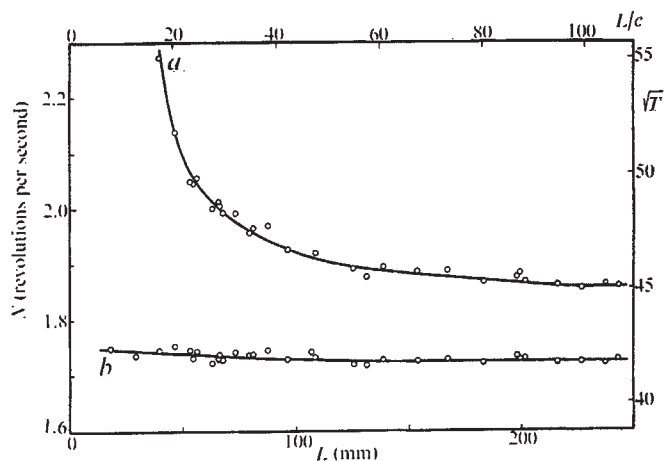


Fig. 2 Effect of annulus length L on critical speeds N determined from torque measurements. a , Wavy vortices; b , Taylor vortices. $R_1/R_2=0.954$.

These visualisation and torque observations at two different cylinder radius ratios agree in indicating little effect of annulus length on the critical speed for the onset of Taylor vortices but considerable effect on the critical speed for the onset of wavy vortices. The length: clearance ratio needed to avoid a rise of more than say 5% in the second critical speed is about 35 but more measurements are required before a precise estimate can be made. Additional preliminary experiments suggested that a similar limit applies when the cylinders are eccentrically positioned, eccentricity raising both critical speeds. Evidently the assumption of infinite length in stability theory is far less satisfactory for the wave instability than for the Taylor instability, and the observation by Coles⁶ on the relationship between the two critical speeds requires re-examination in terms of length effects.

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Table 1 Dimensionless critical speeds
 $\sqrt{T} = [(2\pi N R_1 c)/\nu] \sqrt{[2c/(R_1 + R_2)]}$

Fig. no.	1	2
Experimental method	Visualisation	Torque measurement
Cylinder radius ratio (R_1/R_2)	0.894	0.954
Taylor vortex onset speed:		
Observed $\sqrt{T_c}$	42.7 at $L/c=45.1$	41.9 at $L/c=106.6$
Calculated $\sqrt{T_c}$	42.8 at $L/c=\infty^*$	41.8 at $L/c=\infty^*$
Wavy vortex onset speed:		
Observed $\sqrt{T_1}$	64.0 at $L/c=9.8$	55.1 at $L/c=17.3$
	47.7 at $L/c=45.1$	45.2 at $L/c=106.6$
Calculated $\sqrt{T_1}$	45.8 at $L/c=\infty^\dagger$	44.0 at $L/c=\infty^\ddagger$

*By interpolation from Walowit, Tsao and Di Prima².

†By interpolation from Eagles⁴ (with $m=1$).

‡By extrapolation (so value suspect) from Eagles⁴.

Early turbulence and drag reduction phenomena in larger pipes

SOLUTIONS of certain high molecular weight polymers have been shown to exhibit the phenomena of both early turbulence¹⁻⁷ and drag reduction^{4,5,8} depending on whether the measurements were made under laminar flow conditions for the former case or under turbulent flow conditions for the latter. In general, early turbulence has been observed only in capillary tube flows while drag reduction has been observed in a variety of flow geometries, the only requirement being a developing or developed turbulent flow field.

A correspondence of sorts between early turbulence and drag reduction was surmised earlier⁹ and was also strongly implied by capillary tube measurements where a continuous transition between early turbulence onset points and drag reduction onset had been observed. A close relationship between the two phenomena was therefore suggested⁵. Specifically, the non-Newtonian onset point (either early turbulence or drag reduction) for 1 p.p.m. solutions of Polyox coagulant in a series of salt solutions were found to be described by the following empirical relationship in these capillary flows

$$\tau^* = (78.7)/(1 - 1.39M)$$

where τ^* is the non-Newtonian onset point and M the molarity of the magnesium sulphate salt solution.

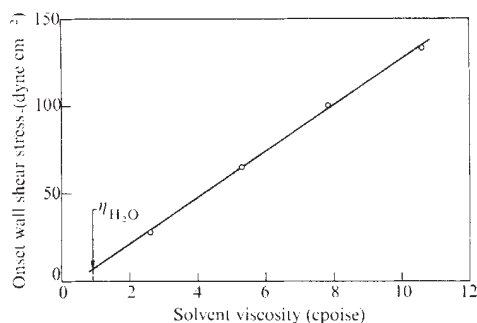


Fig. 1 Onset of early turbulence for 100 p.p.m. WSR-205 solutions in glycerine-water mixtures. Pipe diameter, 0.553 cm.

The suggestion has been made² that early turbulence might also be observed for flows in larger diameter tubes provided sufficiently high wall shear stresses could be attained in the laminar flow regimes. This hypothesis was confirmed by subsequent experiments in larger diameter pipes with drag reducing additives dissolved in water-glycerine mixtures^{6,7}; the glycerine serving the function of significantly altering the solution viscosity, thus providing the needed higher wall shear stresses in laminar flow conditions. Figure 1 shows the variation of the onset wall shear stress for early turbulence with solvent viscosity for 100 p.p.m. WSR-205 solutions in glycerine-water mixtures in a 0.553 cm pipe. The plot is essentially linear over the range of solvent viscosities chosen. The linear relationship suggests that an extrapolation might justifiably be made to the pure water case. Thus, a hypothetical value of 7.4 dyne cm⁻² is obtained for the onset of early turbulence in this particular pipe for the water case had the flow not undergone transition to the turbulent regime. Data⁸ are also available for the same WSR-205 sample under turbulent flow conditions in pure water. Figure 2 shows a plot of the drag reduction onset wall shear stress against the log concentration. Although 100 p.p.m. WSR-205 had not been studied in the earlier work a short extrapolation

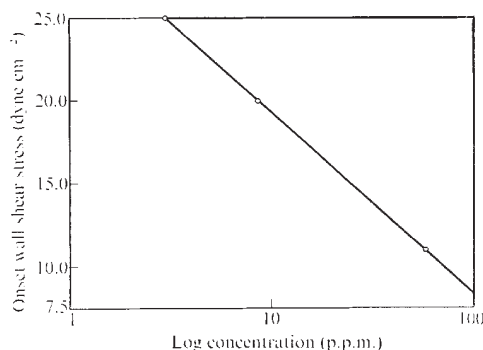


Fig. 2 Onset of drag reduction against log concentration for WSR-205 solutions in turbulent flow. Pipe diameter, 0.660 cm.

of the linear plot in Fig. 2 to the 100 p.p.m. value yields an onset wall shear stress for drag reduction of approximately 8 dyne cm⁻². Although both sets of data rely on short extrapolations this additional evidence does seem to support earlier claims^{4,6,9} that drag reduction and early turbulence are somewhat related phenomena.

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Preserved amino acids from silicified protein in fossil Radiolaria

THE discovery of amino acids in fossils¹ has stimulated a wide range of biochemical studies^{2,3} over the past 20 yr with implications for evolution, geochronology, and biomineralisation. One productive line of evolutionary investigation has been the analysis of calcified protein from Recent shells of calcareous invertebrates such as molluscs^{4,5} and planktonic Foraminifera^{6,7}. Results indicate that the amino acid composition is species-specific and varies in a systematic manner which parallels morphology. Moreover, data from extinct 18-Myr-old Foraminifera suggest that the technique can be applied directly to the deep-sea fossil record for establishing phylogenetic affinities^{6,7}. Virtually nothing is known, however, of the nature of the organic matrices which may exist in fossil skeletons composed of silica rather than calcium carbonate. Amino acid data from this study are therefore believed to be the first evidence reported for the presence and diagenesis of silicified protein in the fossil record.

Radiolaria, important contributors to the siliceous ooze characteristic of Equatorial Pacific and Antarctic regions, were

Table 1 Amino acid composition of Recent skeletal protein*

Amino acid	Recent Radiolaria (silicified)			Mean	Recent Foraminifera (calcified) ⁷ Mean of 16 species
	<i>S. antarctica</i> <i>cum</i>	<i>S. glacialis</i>	<i>C. neatum</i>		
Asp	151	154	167	156	212
Thr	50	40	43	44	82
Ser	80	40	70	63	84
Glu	115	135	134	127	114
Gly	193	299	192	227	138
Ala	88	81	100	90	100
Val	82	48	82	71	60
Met	6	3	<1	5	9
Allo-Ileu	4	3	3	3	—
Ileu	29	25	28	27	36
Leu	50	58	68	59	44
Tyr	13	11	20	15	23
Phe	31	30	30	30	35
His	1	3	<1	2	20
Orn	10	5	11	9	—
Lys	53	49	14	39	22
Arg	44	16	38	33	21
Total Concentration	(1,355)	(1,602)	(1,360)	(1,439)	(2,523)
(nmol/g skeleton)					

* Residues per 1,000 amino acid residues.

Core samples were disaggregated in a dilute sodium hexametaphosphate solution with ultrasonic vibration, washed with distilled water on a 62 µm sieve, and air dried. Although the skeletons of these planktonic protozoans are generally small (50–500 µm), certain species were found to be sufficiently large and abundant to allow manual extraction from the coarse fraction under a binocular microscope. Samples from aggregate species were exhaustively cleaned ultrasonically in a series of sodium hexametaphosphate washes and distilled water rinses, dissolved in cold hydrofluoric acid, evaporated to dryness, and hydrolysed in 6 N HCl in sealed tubes under nitrogen at 110°C for 22 h. Hydrolysates were evaporated to dryness in a vacuum centrifuge and stored in a freezer until analysed. Suitable species were not found in sufficient abundance for replicate analyses and results are therefore based on single analyses. Analyses of sub-mg monospecific collections of low-density skeletons (2–5 µg per skeleton) were made possible with a Durrum D-500 amino acid analyser operated in manual mode at the pmol (~10⁻¹⁰ g) level of sensitivity. Reproducibility is within ± 5% based on replicate analyses of standard amino acid mixtures. Results in Tables 1 and 2 are corrected for reagent blank values and hydrolytic losses of Thr and Ser.

the first group of silica-secreting organisms chosen for study. All species analysed are members of the Polycystina group which are currently defined as "Radiolaria with skeletons of opaline silica without admixed organic compounds"⁸, based on earlier microscopic evidence^{9,10}. Before this study, only the Tripylea group was thought to contain skeletal organic matter. Tripyleans are rarely preserved in deep-sea sediments because of their fragile structure.

Two of the three Recent species analysed (*Spongoplegma antarcticum* Haeckel and *Spongostrochus glacialis* Popofsky) were isolated from the top of an Antarctic deep-sea core (RC12-229), the third (*Cyclampterium neatum* Sanfilippo and Riedel) from the top of an Equatorial Pacific core (RC12-66). Down-core species (*Cyclampterium neatum* and *Dictyastrum angulatum* Ehrenberg) were separated from RC12-66 at the palaeomagnetic boundary of Gilbert/Epoch 5 which corresponds to an age of 5.1 Myr.

A protein-containing organic matrix was found in all three species of Recent Radiolaria (Table 1). With the exception of cystine, all amino acids normally found in protein hydrolysates were present. Proline was detected, but not quantified, with the 590-nm photometer in the analyser. Two additional amino acids, D-alloisoleucine and ornithine, were found in significant amounts indicating that some diagenesis had occurred in the few thousand years represented by the core top samples.

Each species has a unique composition with inter-species variations being greater than experimental error for 16 of the 17 amino acids. Glycine, aspartic acid, glutamic acid, and alanine are the four most abundant amino acids in all three species and account for 60% of all residues.

The only notable difference between the two spumellarian species and the single nassellarian is a 73% lower abundance of lysine in the nassellarian skeletons. The Spumellaria and the Nassellaria are the two major taxonomic groups within the Order Polycystina⁸.

Total amino acid concentration in silicified radiolarian skeletons is about half the content of calcified foraminiferal shells (Table 1). Significant differences are also observed in the proportion of certain amino acids although the four most abundant acids in both mineral types are glycine, aspartic acid, glutamic acid, and alanine. The relative abundance of aspartic and glycine is reversed in the two groups with glycine being greater in Radiolaria (aspartic second), whereas aspartic acid ranks first in

Table 2 Diagenetic changes in amino acid content of radiolarian skeletons over 5.1 Myr

Amino acid	Spumellaria			Nassellaria		
	Recent <i>S. glacialis</i> (nmol g ⁻¹)	5.1 Myr <i>D. angulatum</i> (nmol g ⁻¹)	Ratio 5.1 Myr/Recent	Recent <i>C. neatum</i> (nmol g ⁻¹)	5.1 Myr <i>C. neatum</i> (nmol g ⁻¹)	Ratio 5.1 Myr/Recent
Asp	246.4	27.4	0.11	226.9	10.8	0.05
Thr	64.8	—	—	58.4	5.0	0.09
Ser	64.0	9.7	0.15	95.0	13.9	0.15
Glu	216.8	81.4	0.38	181.9	24.3	0.13
Gly	477.6	145.7	0.31	260.9	31.6	0.12
Ala	129.3	93.0	0.72	135.3	25.8	0.19
Val	76.5	57.6	0.75	111.9	12.5	0.11
Met	5.3	—	—	Trace	—	—
Allo-Ile	5.3	11.2	2.11	4.4	1.9	0.43
Ile	39.6	16.4	0.41	38.4	2.7	0.07
Leu	93.5	27.9	0.30	92.2	11.8	0.13
Tyr	17.1	—	—	26.6	Trace	—
Phe	48.3	15.3	0.32	40.9	8.8	0.22
His	5.1	—	—	Trace	—	—
Orn	7.8	365.5	46.86	15.3	11.1	0.73
Lys	78.3	8.6	0.11	19.4	3.0	0.15
Arg	26.3	—	—	52.2	—	—
Totals	1,602.0	859.7	0.54	1,359.7	163.2	0.12

Foraminifera (glycine second). Other major compositional differences are 77% more lysine, 46% less threonine, 35% less tyrosine, and 34% more leucine in radiolarian skeletons.

Approximately 75% of the total amino acid content is peptide-bound. Analyses of non-hydrolysed samples of Recent *Spongoplegma antarcticum* and *Spongostochus glacialis* show total 'free' amino acid concentrations amounting to 25% of the hydrolysed values. As Recent planktonic Foraminifera show less than 5% 'free' amino acid content (K.K., unpublished), diagenesis of proteins seems to be accelerated in siliceous skeletons. Experiments with Recent foraminiferal protein indicate that the hydrofluoric acid dissolution step does not contribute significantly to the observed hydrolysis in Radiolaria.

Comparative analyses of samples from Recent and 5.1-Myr-old species (Table 2) indicate that the protein-containing organic matrix undergoes severe diagenesis over geological time. An 88% reduction in amino acid content was found when the 5.1-Myr-old sample of nassellarian *Cyclampterium neatum* was compared with a Recent sample. A substantial reduction (46%) was also observed in the pair of spumellarians. If the diagenetic product, ornithine, is excluded from the comparison, the effect is more similar for the two groups, with reductions of 69% (Spumellaria) and 89% (Nassellaria). Scanning electron micrographs suggest that the substantially higher diagenetic rate in the nassellarian is related to a greater susceptibility to solution because of a nodulose skeletal surface¹¹.

The 'free' amino acid content of a non-hydrolysed 5.1 Myr nassellarian sample represents only 19% of the total content on hydrolysis, whereas 77% of the acids from a hydrolysed spumellarian sample of the same age is accounted for by 'free' acids¹¹. These results suggest that the bulk (80%) of the remaining few amino acids in the nassellarian are in bound form and may represent either well-protected intact protein or humic acid-type polymers from *in situ* reactions¹².

The diagenetic reductions of 69% (Spumellaria) and 89% (Nassellaria) in 5.1-Myr-old samples are in marked contrast to an average reduction of 40% observed in 18-Myr-old Foraminifera^{6,7}. Factors which may contribute to the accelerated diagenetic rate of Radiolaria are (1) the high internal water content (up to 20% by weight) of opaline silica¹³ promoting the hydrolysis of peptide bonds, and (2) greater skeletal porosity leading to more rapid attack and leaching by external pore waters in the sediment.

Contamination of fossil materials by amino acids in the sediment is always a potential problem because of the ubiquitous nature of these compounds. As a test for surface contamination *in situ*, samples of *S. antarcticum* and *S. glacialis* were treated with concentrated (30%) hydrogen peroxide for 1 h before normal sample preparation. No significant differences in composition were observed between these samples and those cleaned with only sodium hexametaphosphate.

The presence of a protein-containing matrix in radiolarian skeletons provides a rationale for extending amino acid studies to siliceous fossils. Promising lines of investigation would include the dating of siliceous sediments by the epimerisation-racemisation reactions of preserved amino acids^{14,15} and the tracing of evolutionary lineages by the analysis of species-specific amino acid patterns⁷.

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Search for selectivity between optical isomers in reactions of polarised positive muons with alanines and octanols

ONE of the most intriguing problems in chemical evolution is the origin of optical asymmetry in biopolymers. The easiest way to state the problem is: why are proteins made almost exclusively of L-amino acid optical isomers and natural sugars of D-optical isomers¹? That proteins must be made of only one kind of optical isomer is understandable on the basis of their need for precise three-dimensional conformations in order to perform their catalytic roles as enzymes. But, is it just a matter of chance, as suggested in refs 2-4, that our proteins are L-, or is there (was there) some asymmetrical agent on our planet that made the protein L-configuration the one upon which life is based?

One possible 'non-chance' explanation stems from the unequal decomposition of optical isomers^{5,6} and the appearance of optical activity in the products of light-mediated reactions⁷⁻¹⁰ in circularly polarised light. A slight excess of right-circularly polarised light in sunlight reflected and/or refracted in the Earth's magnetic field¹¹ could produce overall optical asymmetries. But laboratory experiments have detected such effects only for high intensities of circularly polarised light; this mechanism has therefore been seriously questioned as an explanation for the origin of optical asymmetry in biological molecules¹.

Table 1 Residual muon polarisation in various media

Target	Residual asymmetry (<i>A</i>)	Residual polarisation* (<i>P_{res}</i>)
Distilled water	0.153 ± 0.003	0.55 ± 0.03 (def.)*
0.67 M racemic alanine in water	0.151 ± 0.004	0.54 ± 0.03
Solid L-alanine	0.089 ± 0.003	0.32 ± 0.02
Solid D-alanine	0.089 ± 0.003	0.32 ± 0.02
Liquid L-2-octanol	0.140 ± 0.003	0.50 ± 0.03
Liquid D-2-octanol	0.140 ± 0.002	0.50 ± 0.03

* Residual polarisation derived from $P_{res} = A/A_0$, where $A_0 = 0.278 \pm 0.016$ is calculated by comparing $A(H_2O)$ with the independently determined residual polarisation in water, $P_{res}(H_2O) = 0.55 \pm 0.03$ (ref. 18).

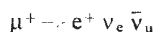
A second possible 'non-chance' explanation invokes spin-polarised electrons and associated bremsstrahlung emitted in the β -decay of certain natural radioactive nuclides^{12,13}. One report claims that in aqueous solution D-tyrosine is decomposed by ⁹⁰Sr β emission faster than L-tyrosine¹⁴. This report has been questioned by later papers^{15,16}, however, and must be considered unsubstantiated.

This paper is concerned with a search for optical selectivity in the interaction of a new agent, the polarised positive muon, with organic molecules. The degree of spin polarisation in muon beams produced from decaying pions is typically about 80% (refs 12 and 13). As far as we are aware, this is a higher degree of polarisation than that of any particles previously used in the search for selectivity of interactions with optical isomers. Since polarised muons are known components of cosmic rays¹⁹, a selective interaction, possibly leading to selective destruction of one optical isomer, might provide another 'non-chance' explanation for the particular appearance of L-amino acids and D-sugars in living cells.

Positive muons, upon slowing down, form the neutral atom 'muonium' ($\mu^+ e^-$, or Mu)²⁰ which is analogous to the hydrogen atom, except for its mass, $m_{\text{Mu}} = 0.1131 m_{\text{H}}$. Because of the high degree of polarisation of the μ^+ 'nucleus', the formation of the muonium atom and/or its subsequent chemical reactions might vary between optical isomers. We have, however, been unable to detect any optical selectivity in the interactions of muonium with either (1) solid D- and L-alanine or (2) liquid D- and L-2-octanol.

The μ^+ beam of the Berkeley 184-inch cyclotron¹⁸ was used in these experiments. The quantity actually measured was the 'residual polarisation' of muons stopped in the various substances. Although the muons enter the target with a well-defined spin polarisation, their polarisation after stopping is dramatically dependent upon the chemical properties of the medium in which they come to rest.

The spin polarisation of the muons is easily detected through their decay products: the muon decays by way of



with a mean lifetime of 2.2 μs . The positron (e^+) from the decay is, on the average, about twice as likely to be emitted in the direction of the μ^+ spin as in the opposite direction^{17,18,21}. An ensemble of polarised positive muons thus broadcasts its polarisation in a shower of fast (up to 50 MeV) positrons. By detecting these positrons in scintillation counters, we monitor the magnitude of the μ^+ polarisation. In the present experiments, a perpendicular magnetic field is applied, causing the μ^+ spin to precess at its Larmor frequency. A counter telescope fixed in the plane of precession is most likely to detect a positron if the muon decays when its spin points towards the telescope; thus the e^+ detection probability in that telescope will rise and fall as the muon polarisation sweeps past it¹⁸.

The measured amplitude of the resultant time oscillation of the positron detection probability is called the experimental asymmetry, A , and is proportional to the residual polarisation of the μ^+ , P_{res}

$$A = A_0 P_{\text{res}}$$

The constant of proportionality A_0 is an empirical constant unrelated to the chemical properties of the medium^{22,23}. The values of P_{res} obtained in the present work are given in Table 1. Since water has been thoroughly studied^{22,23}, the value of P_{res} (H_2O) = 0.55 ± 0.03 from refs 22, 23 is used to calibrate the results listed in Table 1. A more detailed technical description of the apparatus and experimental technique can be found in refs 18, 22 and 23.

Each target consisted of about 500 g of the substance under study. The solid alanines (A grade) were obtained from Calbiochem, La Jolla, California, and the liquid octanols from Norse Laboratories, Santa Barbara, California. All samples were used without further purification.

In most condensed media, any residual polarisation observed through muon precession is the result of chemical reactions of muonium with molecules of the medium; in the absence of such reactions, all the muon polarisation is lost within a few nanoseconds through the so-called 'muonium mechanism'. This depolarisation mechanism arises after the formation of muonium and is due to the contact hyperfine interaction between μ^+ and e^- magnetic moments, which couples the two spins together so as to reverse the μ^+ spin within $\sim 10^{-10}$ s. Fast precession of muonium in the external magnetic field conspires with this hyperfine coupling to effectively depolarise the μ^+ in muonium within $\sim 10^{-9}$ s. The only way a muon can be spared this fate is if the Mu atom that it forms reacts chemically with a molecule of the medium to incorporate the μ^+ into a diamagnetic molecule.

A rigorous theoretical description of the mechanism of μ^+ depolarisation is found in refs 22–24. Briefly, there are two sorts of chemical reactions of Mu that prevent complete depolarisation of the μ^+ : (1) 'normal' thermal reactions, which occur after the Mu atom has thermalised and started depolarising the μ^+ ; and (2) epithermal or 'hot atom' reactions, which occur while the Mu atom is still slowing down, long before any depolarisation has taken place. The latter reactions are assumed to be important in the energy region from ~ 10 eV down to thermal energies, and are usually the dominant channel for reactions of Mu in all but the most reactive substances. In water or methanol, for instance, the residual polarisation is believed to be due exclusively to hot atom reactions^{22,23}.

As can be seen from Table 1, the residual polarisation in a 0.67 M solution of racemic alanines in water is the same, within experimental uncertainties, as that observed in pure water. We conclude that there is little, if any, thermal reaction of Mu with alanine molecules, at least in solution^{22,23}. Taken with the observation that Mu has a large epithermal reaction probability in most organic substances, this suggests that the residual polarisation in the solid alanines is primarily a result of hot atom reactions of muonium. The same assumption can be made for the octanols, on the somewhat weaker grounds that Mu has a large, purely epithermal reaction probability with methanol^{22,23}.

The data in Table 1 show that there is no significant difference between the reaction probabilities of polarised Mu atoms with enantiomers of alanine and 2-octanol. The errors given are derived from the fitting procedure (see refs 22 and 23). Although it is statistically possible that a few per cent difference could be realised ($\Delta A/A = 0.0 \pm 0.05$ for alanine and $\Delta A/A = 0.0 \pm 0.03$ for 2-octanol), this difference could only be determined accurately on the basis of more measurements. Remembering that it is the μ^+ nucleus of the Mu atom that is polarised (negligible polarisation is transmitted to the electron in the time of $\sim 10^{-12}$ s that muonium takes to thermalise), it is perhaps not surprising, in retrospect, that the hot atom reactions are optically indiscriminate.

A more sensitive test of optical selectivity of polarised muons may be possible if muonium precession can be observed directly in these substances. Apparently, recent results using positrons²⁵ suggest that electrons picked off by muons to form Mu atoms might be expected to have an optically-influenced polarisation. This effect would be reflected in first order in the amplitude of muonium precession signals. The feasibility of such studies is now being considered.

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Bilayers at the air–water interface?

FILMS of octadecanol and the corresponding C_{18} acid (stearic acid) appear to occupy 10^2 Å^2 per molecule at the air–water interface when spread from ethanol or acetone, as opposed to 20 Å^2 per molecule when spread from a (non-polar) solvent such as hexane or benzene. Ries¹ has proposed that in a polar or non-polar spreading solvent the amphipathic molecules adopt a bimolecular configuration with the hydrophilic groups pointing, respectively, outward and inward. It was further postulated that when spread at an air–water interface, only the latter could be ‘unzippered’ by the substrate to form a monolayer. Spreading from a polar solvent would thus result in bilayer formation and explain the apparent loss of half the film molecules. In view of the upsurge of interest in membrane and model membrane systems, the possibility of forming a lipid bilayer at the air–water interface was certainly exciting and clearly warranted further investigation.

Films of L- α -dipalmitoyl phosphatidylcholine (DPPC), 1-monolein and stearic acid (Applied Science Laboratories; purities > 99%) were selected for study. DPPC and 1-monolein are examples of substances which do, and do not, readily form a bilayer². Isotherms and surface potentials for spread films of these three substances are shown in Figs 1–3. Shifting from hexane to ethanol resulted in a substantial decrease in the apparent area per molecule for all three films, the extent of the decrease being dependent on the nature of the film molecules when other factors were held constant. The large shift to smaller areas per molecule occurs between 90 and 100 volume per cent ethanol (Fig. 4a). Between 0 and 90 mol per cent ethanol, the

already liquid-expanded isotherms become slightly more expanded at low surface pressures and the surface potentials appear to decrease slightly (Fig. 1), both effects indicating the presence of small amounts of ethanol in the substrate¹. Contrary to Ries¹, the large shift in areas per molecule was sensitive to changes in solute concentration (Fig. 4b) and even to the rate of delivery at the air–water interface. In effect, a 50% decrease in apparent area per molecule can be obtained simply by selecting the correct spreading solute concentration for that particular component. Of particular interest, is the surface potential which showed little change on transition from monolayer to ‘bilayer’. In addition both phase changes and compressibility are independent of the apparent physical state of the film. These latter observations led us to question seriously the assigned bilayer configuration for films spread with polar solvent and to attempt to respread an original stearic acid ‘bilayer’ as a monolayer using a non-polar solvent.

The respread isotherms initially revealed gross contamination and great care was required before hexane-respread films (originally spread either from hexane or ethanol) were impurity free. When

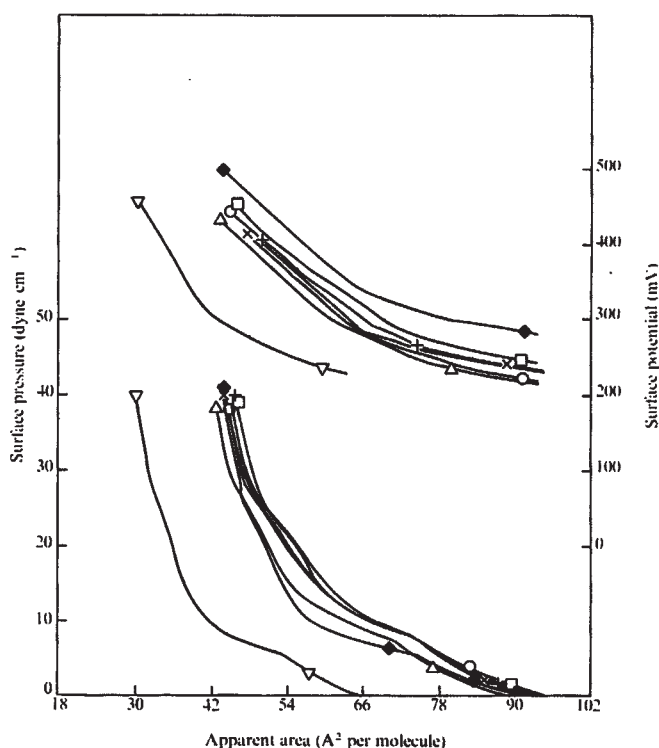


Fig. 1 L- α -Dipalmitoyl phosphatidylcholine (DPPC) isotherms at the air–water interface (pH 6 substrate) at 22° C. Films spread from various mixtures of ethanol and hexane. Percentage ethanol: $\blacklozenge$, 10; $\square$, 44; $\circ$, 50; $\times$, 60; $+$, 70; $\triangle$, 90; ∇ , 100. Isotherms and surface potentials were continuously recorded. The upper curves are the surface potentials. Spreading solvent concentration approximately 0.15 mg DPPC per ml solvent. Ethanol and hexane were column purified and distilled before use. Hydrochloric acid used in the aqueous substrate was also distilled. Water was double-distilled from glass (the first time from alkaline permanganate) and subsequently double-distilled from quartz. An automated continuous recording teflon trough film-balance was used, which together with initial film spreading techniques, has been described elsewhere³. Techniques were developed for the removal, concentration, solution and respreading of initially spread films after it was found that adding solvent to a spread monolayer or bilayer clearly indicated solvent retention. Scrupulous care in cleaning glassware, trough and solvents were required for reproducibility in such experiments. Reproducibility for initially spread films was $\pm 0.5 \text{ Å}^2$ per molecule for a given surface pressure (π) and $\pm 10 \text{ mV}$ for surface potential values. Reproducibility in areas per molecule on respreading from ethanol a film initially spread from hexane was approximately $\pm 5\%$ at a value of 15% less than the originally spread film. The initial spread film was never compressed beyond 15 dyne cm^{-1} . Respreading films with hexane, originally spread from ethanol, gave values with some 10% or less film loss.

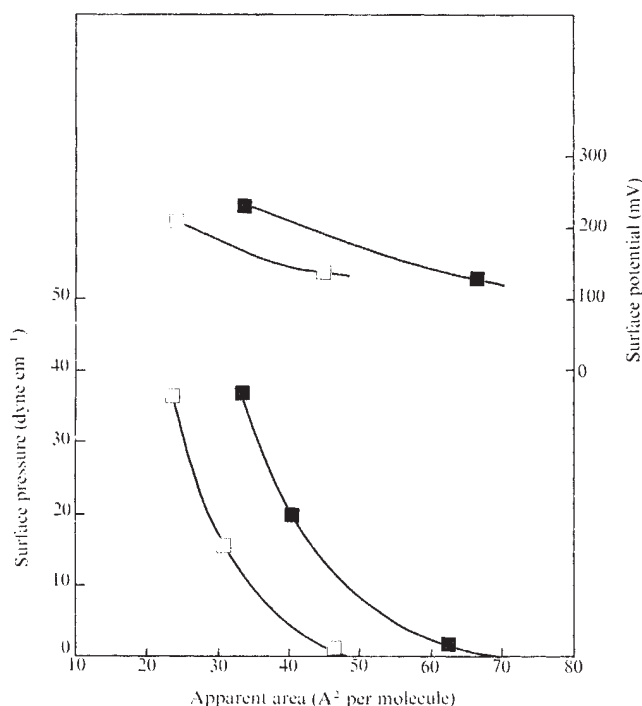


Fig. 2 1-Monolein isotherms at the air-water interface spread from ethanol (□) and hexane (■) at 21.5° C. (pH 6 substrate). Spreading solvent concentration approximately 0.1 mg 1-monolein per ml solvent.

this was achieved (Fig. 3), respread areas per molecule were usually slightly lower (~10%) than the original film values. For films previously spread from ethanol, slightly high areas per molecule were obtained by removing significant amounts of the aqueous substrate with the original film. Only when high solute concentrations were used, were areas per molecule obtained for a respread film that were slightly greater than those for the original 'bilayer'. Immediately after film removal the substrate appeared clean, but after an hour or so, however, trace amounts of stearic acid were once again obtained. These additional amounts did not arise from collapsed material

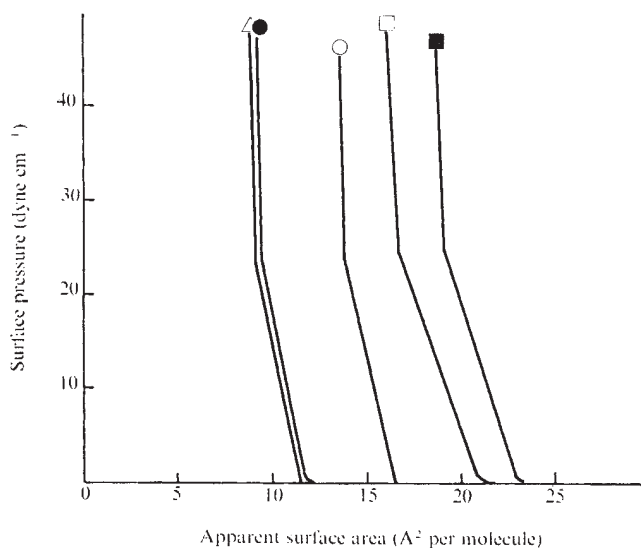


Fig. 3 Stearic acid films at the air-water interface spread from hexane (■) and respread with hexane (□); from ethanol (●) and respread with hexane (△); from ethanol at pH 2 (○). Substrate at pH 6 except where indicated. Initial spreading solvent concentration approximately 0.21 mg stearic acid per ml solvent.

at the trough edge. Finally, addition of acid to the substrate before initial spreading from ethanol led to areas per molecule intermediate between those of a 'bilayer' and a monolayer (Fig. 3). A similar, though smaller, effect was obtained by adding salt to the substrate.

From the above, we conclude that spreading of a film with a polar solvent does not result in the formation of a bilayer, but in fact leads to the loss of a significant amount of material, the precise amount depending on the nature of the film material, spreading solvent, the substrate pH and ionic strength, and also on the spreading solvent concentration and rate of deposition. The effect of the substrate additives indicates that film loss occurs through the substrate.

We have observed two phenomena which help explain these results: (1) Substantial film loss is accompanied by gross Teflon trough contamination. A clean trough permits rapid, complete water drainage. After multiple ethanol depositions and film

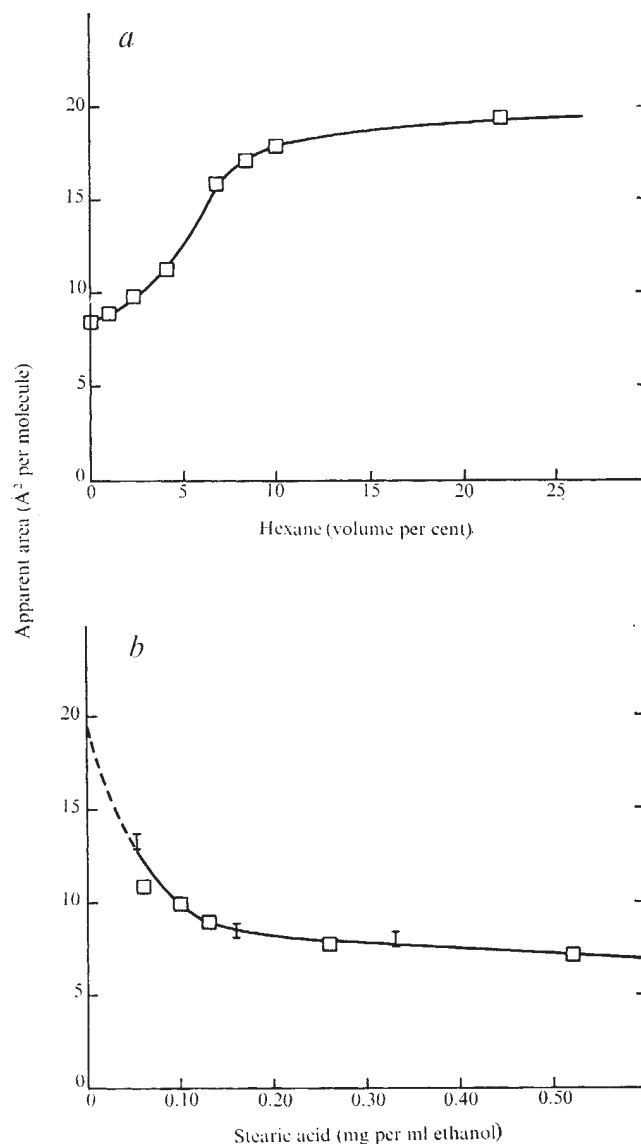


Fig. 4 *a*, Apparent area per molecule of stearic acid as a function of spreading solvent composition. A 0.29 mg stearic acid per ml ethanol solution was diluted with hexane to give the stated ethanol-hexane ratio prior to spreading at 20.5° C. *b*, Apparent area per molecule of stearic acid as a function of its concentration in ethanol at 20.5° C. The zero abscissa value was obtained by spreading from hexane. Points marked (I) were obtained by at least three determinations. Points marked (□) were obtained by preparing the most concentrated solution and then carrying out a series of dilutions.

loss, water film retention on the trough was greater than normal. (2) Schlieren patterns are readily observed during ethanol spreading at the air-water interface, penetrating deeply into the water. This behaviour is greatly reduced by the addition of small amounts of hexane. We postulate, therefore, that most of the lost film material is trapped at the Teflon surface. The well known porosity of Teflon provides a substantial surface area for this purpose. Certainly it is not located at the air-water interface.

We conclude that spreading from a polar solvent results in substantial film loss but that the residual film is still a monolayer; the near identical surface potential, phase changes and compressibility, independent of spreading solvent, all support this conclusion.

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***In vivo* nuclear fission in the aetiology of decompression sickness**

THERE can be little doubt that gas bubbles accompany and may well cause decompression sickness, as the effect of recompression is usually to alleviate the symptoms¹ and to eliminate the new reflectors of ultrasound which appear in man and animals during decompression²⁻⁵.

As gas bubbles do not form in supersaturated fluids or animals without gas micronuclei^{6,7} we assume that the body of a normal man must contain microbubbles, which may expand at decompression to give the observed effects. Furthermore, it seems that such microbubbles must be created continually⁷. Some studies of bubble formation *in vivo*⁸⁻¹⁰ assume implicitly the presence of pre-existing micronuclei, which evades the problem.

One mechanism for the production of micronuclei in man, tribonucleation, has been suggested by Ikels¹¹. We suggest here that some gas micronuclei are created in man by spontaneous nuclear fission (SF) *in vivo*. Ionising particles dissipate energy along their tracks and if the linear

energy transfer (LET) to a metastable liquid is sufficiently high, the resultant 'thermal spike'¹² may nucleate bubbles which can subsequently grow to visible size, as in the bubble chamber.

Water is rarely used in bubble chambers¹³, though persistent microbubbles should be created in cold water which is sufficiently supersaturated with gas and subjected to radiation.

As we were unable to find any report of radiation-induced nucleation of aqueous solutions supersaturated with gas leading to bubbles of visible size, we have studied the stability of solutions of two α -active radioisotopes in cold, supersaturated water.

Polypropylene outer containers from Brunswick disposable 1 ml syringes (capacity 12 ml) were filled with stabilised distilled water saturated with air at 215 pound inch⁻² gauge (14.7 bar), (see ref. 15). The top 2 ml of liquid was removed from each tube, 1 ml of solution (Table 1) was introduced, and the tube was topped up with liquid paraffin and sealed by a thin polythene sheet. The tubes then received high hydrostatic pressure treatment to eliminate any gas micronuclei introduced with the additions. The final gas tension in the amended tubes was about 13.1 bar. The tubes were then removed for observation at atmospheric pressure for 6 h. If visible bubbles appeared in any tube during this period it was recorded as unstable; the other specimens were recorded as stable.

Most of the control tubes remained stable for the full 6 h and the tubes with the lead salt were no less stable than the controls (Table 1). We may, therefore, conclude that a heavy metal ion does not necessarily promote bubble formation. The tubes containing uranium were, however, much less stable than the controls, the difference being statistically significant ($\chi^2=208$, $P<0.0005$).

To distinguish between the effects of the principal mode of radioactive decay for ²³⁵U (α emission) and the much less frequent spontaneous nuclear fission, thorium tubes were prepared. The quantity of salt was arranged so that the α emission was the same as in the uranium tubes, and the α energies are similar. The SF half-life of ²³²Th (ref. 16) is so much greater than that for ²³⁵U (ref. 17) that the probability of one event for all 100 thorium tubes in 6 h is less than 10⁻².

The thorium tubes were only marginally less stable than the controls (Table 1): the difference between the thorium and uranium results is again highly significant ($\chi^2=93$, $P<0.0005$). We conclude, therefore, that the nuclear fragments which are liberated by SF of ²³⁵U will nucleate bubbles which can grow to visible size in cold supersaturated water.

It may not be widely appreciated that spontaneous nuclear fission occurs in man. The phenomenon seems to occur, however, sufficiently often to maintain a stock of stable gas micronuclei in an average man, and exceptionally it may promote bubble formation during one decompression.

The principal isotope must be ²³⁵U, of which the body of an average man in the UK contains about 110 μ g (ref. 18).

Table 1 Proportion of stable specimens of water supersaturated with air at 13.1 atm with added solutions

	6-h results		Weight of addition		α activity		SF	SF
	Stable proportion	Stable (%)	Salt	Isotope	pCi	dis s ⁻¹	Disintegration h ⁻¹	Disintegration (600 h) ⁻¹
Control (water)	262/287	91.3	none					
Pb(NO ₃) ₂	95/100	95.0	1,000 mg					
²³⁵ U*	56/200	28.0	8 mg	4.6 mg	1.5	56	9.2×10^{-2}	55
²³² Th†	87/100	87.0	41 mg	13.6 mg	1.5	56	$<2.8 \times 10^{-6}$	$<1.6 \times 10^{-3}$

* Uranium oxalate. † Thorium nitrate.

If the SF half-life of ^{238}U is 10^{16} yr (ref. 17) man should experience a disintegration about once every three weeks.

A new nucleus might not survive compression. But if each disintegration during decompression, or during the preceding period at raised ambient pressure were to cause an attack of decompression sickness, a large group of men working eight-hour shifts at pressure should experience a bends rate of about 2.2% of man-decompressions. At the Tyne Road Tunnel men were working for some months at pressures of 27–29 pound inch $^{-2}$ gauge. Of the 5,465 man-decompressions recorded after shifts of 8 h or more at these pressures, some 118 resulted in an attack of bends¹⁹, giving a rate of 2.16%.

One mechanism for the stabilisation of new nuclei is an 'organic skin'^{20,21}, though in our case high temperatures are involved so fission-induced cavities may well be surrounded by a coagulum²² of denatured protein. Certainly, an abundant supply of suitable macromolecules is to be found in most body fluids. The body burden of uranium resides mostly in bone^{18,23}. The commonest symptom of acute decompression sickness (the bends) is described as a diffuse pain associated with bones, and the most important chronic consequence of hyperbaric exposure is Caisson disease of bone. In dogs, the ends of the long bones retain more uranium than the shaft²³, and both in dog²³ and in man²⁴ the element is mainly found on the surfaces of cancellous bone and the endosteum. Both are sites associated with the lesions of Caisson disease of the femur and humerus.

There can be considerable geographical variation in the total α activities of human bone ash samples²⁵. If a similar variability in the uranium content—and therefore in the formation rate of fission-induced nuclei—could be discovered and correlated with the observed variation in individual susceptibility to decompression sickness, it may become possible to prove or refute our hypothesis. From our comprehensive records of diving experience and work in compressed air²⁶ it is easy to select men who are particularly resistant or susceptible, though estimation of the body burden of uranium in live men without occupational exposure is less easy. The traditional method is to determine the uranium content of urine^{27,28}, and despite uncertainty in relating the excretion rate to body burden, it seems probable that body burdens may be compared usefully in this way.

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Pleistocene date for man in Tasmania

THE Pleistocene colonisation of Tasmania has long been predicated^{1,2} but no dated human occupation sites of that age have been reported before. The excavation of a cave site on Hunter Island³, 6 km off the coast of north-western Tasmania (40° 34'S, 144° 45'E; Fig. 1) has yielded the first radiocarbon date of Pleistocene age from a human occupation site in Tasmania.

The Cave Bay Cave is a large sea cave in a slate cliff containing an undisturbed deposit rich in faunal remains and with signs of human occupation. In a preliminary cutting 1.75 m deep, the top 25 cm were found to contain abundant evidence of man: hearths, lenses of marine shell and quartz flakes were present, and the bones of seabirds predominated. Below this level, evidence of human occupation was sparse. The deposit was still rich in faunal remains but seabirds were absent whereas native rodents and small marsupials were

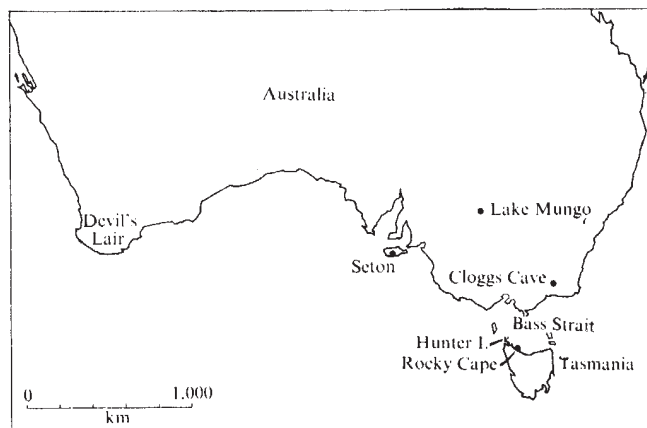


Fig. 1 Southern Australia.

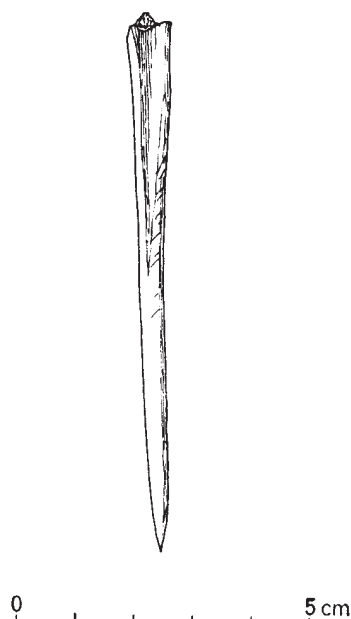


Fig. 2 Bone point found in Pleistocene deposits in Cave Bay Cave.

abundant. There were also some larger marsupials, some of which are not now found on Hunter Island, including the native cat (*Dasyurus* cf. *viverrinus*). A bone point and two pieces of flaked quartz—convincing evidence of the presence of man—were found between 80 and 100 cm below the surface. The bone point is a ground and polished piece of macropod fibula (Fig. 2). Associated charcoal has been carbon dated to $18,550 \pm 600$ b.p. (ANU-1361).

The final maximum of the last glaciation was responsible for lowered sea levels about 18,000 yr BP and Hunter Island would have been a hill on a land bridge between Tasmania and the Australian mainland⁴.

Similar sites in southern Australia with Pleistocene dates, bone artefacts and good faunal preservation are Clogg's Cave at Buchan in south-eastern Australia ($17,720 \pm 840$ b.p., ANU-1044)⁵, the Seton site on Kangaroo Island, South Australia ($16,100 \pm 1,000$ b.p., ANU-1221; ref. 6 and R. J. Lampert, personal communication), and the Devil's Lair, Western Australia ($24,600 \pm 800$ b.p., SUA-31)⁷. All of these sites have been the haunt of non-human predators who have contributed their own share of faunal remains. In the Cave Bay Cave, the abundant remains of rodents and small marsupials in the lower levels were probably deposited as pellets regurgitated by owls, and the native cat has probably contributed the bones of larger animals such as bandicoots and possums, which show distinct signs of non-human gnawing.

At Clogg's Cave, the Seton site and the Devil's Lair, there is evidence of the presence of the Tasmanian wolf and devil (*Thylacinus cynocephalus* and *Sarcophilus harrisii*) although there is so far no sign of them at the Cave Bay Cave. The immediate difficulty generated by the presence of these carnivores is that of discriminating the food remains of man from those of the other predators.

A more intriguing problem is suggested by a comparison between these sites and post-Pleistocene cave sites; for instance, the Rocky Cape sites of north-western Tasmania¹. There, a total of 6 m of shell midden deposit has built up over the last 8,000 yr and there is no evidence of the presence or action of predators other than man (ref. 1 and Rhys Jones, personal communication). There is generally much sparser evidence of human occupation at the older sites in terms of artefacts and stratigraphic features than at Rocky Cape. It seems that

Pleistocene man in southern Australia made less intensive use of cave and shelter sites than his post-Pleistocene descendants. Another important human occupation site of Pleistocene age and with faunal remains preserved is at Lake Mungo—an open site where people camped on the beaches of a lake⁸.

The Cave Bay Cave has implications not only for an understanding of the ancestry and development of the unique Tasmanian culture², but also for the ecology of Pleistocene man in Australia.

This investigation is part of a project supported by the Australian National University³.

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Identification of metabolic dechlorination of highly chlorinated biphenyl in rabbit

CHLOROBIPHENYLS with one to four chlorine atoms per molecule are metabolised by certain animals, plants and microorganisms but the more highly chlorinated biphenyls are rather resistant to metabolic change¹.

Organochlorine compounds may be identified at low concentrations in crude extracts of natural samples by a high resolution mass spectrometric method involving photoplate detection²⁻⁴. The method is based on the facts that first, in conditions of high resolution each ion of unique molecular composition is recorded individually and, second, the relatively large mass deficiencies (the actual mass being lower than the closest nominal mass) of both chlorine isotopes. The accurate mass for ³⁵Cl for instance is 34.9688 which accounts for a mass deficiency of -31.1×10^{-3} mass units (m.u.), the corresponding value for ³⁷Cl being -34.1×10^{-3} m.u. These mass deficiencies cause the exact mass of ions which contain these atoms to be at lower masses than ions of the same nominal mass which contain only atoms (that is C, H, N, O) present in most biological molecules. Thus, the high resolution photoplate technique is capable of detecting chlorine-containing mass deficient ions in crude biological extracts. As the photoplate acts as an integrating ion detector it is possible to identify compounds present in low concentrations (≤ 0.01 p.p.m.).

This method has now been applied to the unambiguous identification of metabolites from organochlorine compounds⁵ including the detection of trace components. After the usual dosing of the animal, urine, faeces or organs to be examined were extracted with a suitable solvent and a small portion (10-20 μ g) of the dried extract was placed in the mass spectrometer sample tubes. Mass spectra from the sample were then obtained by exposing photoplates to ions derived from the crude extract for up to 30 min or

longer, each at different temperatures (fractional distillation). The developed photoplates were then screened for metabolites (mass deficient ions) manually or by using an automatic photoplate reader coupled with a computer.

Rabbits were fed a diet of food pellets containing 1% 2,2',4,4',5,5'-hexachlorobiphenyl. The feeding time was 7 d and the total concentration of chlorobiphenyl administered was 1 g kg^{-1} . The urine, which was collected during the entire feeding period, was extracted with ether and the extract treated in the manner described earlier. Manually measured data (perfluorokerosene as internal standard, experimental values $< 10 \text{ p.p.m.}$ from calculated values) showed the presence of two major metabolites (Fig. 1, I, II) formed from the hexachlorobiphenyl by hydroxylation and hydroxylation with concomitant dechlorination respectively. A third, minor, metabolite corresponding to a molecular composition III was detectable only in a limited number of photoplate exposures at about 60°C .

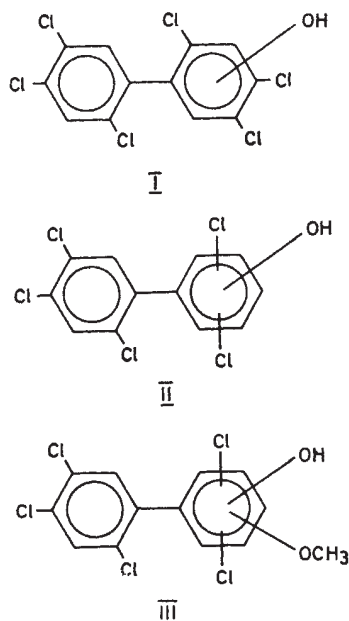


Fig. 1 Metabolites of 2,2',4,4',5,5'-hexachlorobiphenyl.

Figure 2 shows the partial mass spectrum (enlarged photoplate record) of the crude ether extract exposed for 10 min at 60°C . The very mass deficient ions ($> 0.1 \text{ m.u.}$) corresponding to the organochlorine metabolites are seen, clearly resolved at lower mass in each cluster of lines corresponding to ions of the same nominal mass. At m/e 370 and m/e 372 the molecular ion (M^+ ; that is molecular weight calculated with ^{35}Cl) and $M+2$ ion for compound III are visible. Measurement of the exact mass of the ^{37}Cl isotope peak ($M+2$ ion) further confirmed the composition of the compound. At m/e 374 in addition to the weak $M+4$ peak of compound III a strong line corresponding to compound I is evident. At m/e 375 and m/e 376 lines for the ^{13}C and ^{37}Cl isotope peaks of I are obvious.

The advantages of this method in the study of metabolism of organochlorine compounds seem to be first, that no radioactive labelling is necessary because the chlorine atoms act as tracer, second, the method is simple and rapid with unambiguous results so that large scale screening is possible, and third, the danger of loss of metabolite or degradation during separation procedures is minimised and non-volatile products can be detected much more readily than by GC-MS. The usefulness of the method may be con-

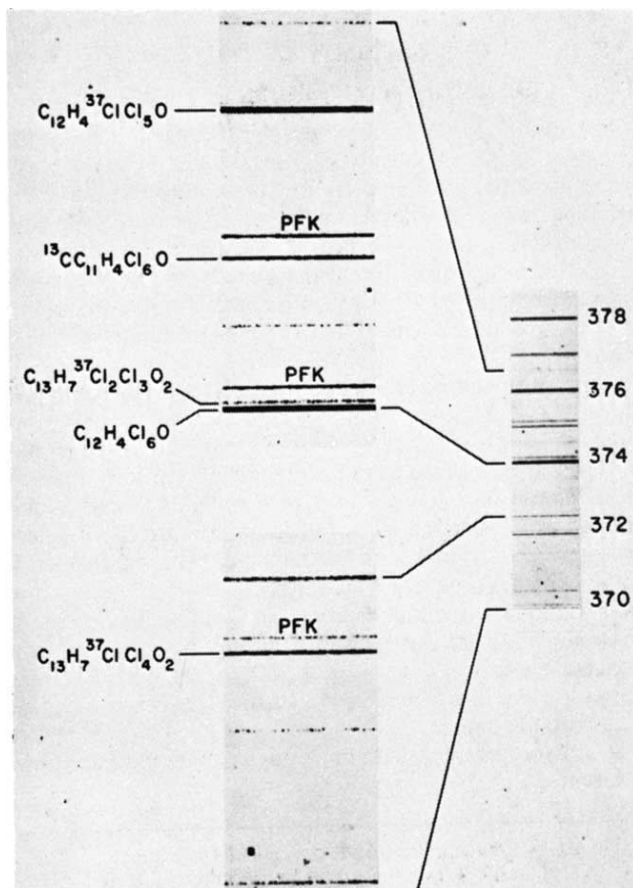


Fig. 2 Partial mass spectrum (m/e 370-376; enlargement of photoplate record) of crude ether extract from rabbit urine. Animals were dosed with 2,2',4,4',5,5'-hexachlorobiphenyl. Lines corresponding to compound III are $\text{C}_{13}\text{H}_7\text{Cl}_3\text{O}_2(M^+; m/e 370)$; $\text{C}_{13}\text{H}_7^{37}\text{Cl}_2\text{Cl}_3\text{O}_2(M^++2; m/e 372)$. Lines corresponding to compound I are $\text{C}_{12}\text{H}_4\text{Cl}_6\text{O}(M^+; m/e 374)$; $\text{C}_{12}\text{H}_4^{37}\text{Cl}_5\text{Cl}_6\text{O}(M^++2; m/e 376)$; $^{13}\text{CC}_{11}\text{H}_4\text{Cl}_6\text{O}(^{13}\text{C}$ isotope peak of $M^+; m/e 375)$. Lines marked with PFK correspond to ions derived from the mass-marker perfluorokerosene. All unmarked lines correspond to mass positive ions from coextracted natural products.

siderably improved in this respect by the use of field desorption techniques.

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Variation in assortative mating in two colonies of Arctic skuas

THE Arctic skua is a polymorphic seabird with pale, intermediate and dark plumaged phenotypes. Sexual selection of the phenotypes takes place when pairs are being formed; during the breeding season the darker males generally mate before the paler males^{1,2}. This gives them a selective advantage because a pair which breeds earlier in the season will fledge a greater average number of chicks^{1,3}. Computer models have been used to estimate the females' mating preferences for the darker males³.

Assortative mating also takes place between the phenotypes. In previously published data⁴, which were taken from observations on several colonies by different ornithologists, the intermediates and darks were not distinguished from each other and were lumped into a single class of dark birds. Some colonies showed assortative mating with a significant excess of pale $\times$ pale and dark $\times$ dark matings compared with the frequencies to be expected if pairing were random. Other colonies showed no deviation from random mating. These differences in assortative mating are not statistically significant, however: an orthogonal analysis gives $\chi^2 = 10.09$ with 6 degrees of freedom corresponding to $P = 0.13$. In this paper we give new evidence of assortative mating, showing that in one colony pale birds mate assortatively while in another intermediates mate assortatively.

Table 1 Observed numbers and theoretical frequencies of matings of Arctic skuas on Fair Isle and Foula in Shetland

Mating type	Observed no. of matings		Theoretical frequencies
	Fair Isle	Foula	
Pale $\times$ pale	7	23	$au + [(u^2(1-\alpha)^2)/(1-au-\beta v-\gamma w)]$
Pale $\times$ inter	24	30	$2uv(1-\alpha)(1-\beta)/(1-au-\beta v-\gamma w)$
Pale $\times$ dark	12	22	$2uw(1-\alpha)(1-\gamma)/(1-au-\beta v-\gamma w)$
Inter $\times$ inter	48	42	$\beta v + [v^2(1-\beta)^2/(1-au-\beta v-\gamma w)]$
Inter $\times$ dark	17	38	$2vw(1-\beta)(1-\gamma)/(1-au-\beta v-\gamma w)$
Dark $\times$ dark	8	10	$\gamma w + [w^2(1-\gamma)^2/(1-au-\beta v-\gamma w)]$
Total no.	116	165	1

To derive the theoretical frequencies, it is assumed in the model that the pale, intermediate and dark phenotypes occur in the proportions u , v and w and mate assortatively with another bird of the same phenotype in the proportions α , β and γ . Thus the proportion of all birds that mate at random is $1-au-\beta v-\gamma w$.

Research on the colony of Arctic skuas on Fair Isle began in 1948 and continued until 1962. In 1973 we started further researches in order to analyse the components of natural and sexual selection which the earlier research had revealed. In 1974 we also surveyed the Arctic skuas on Foula, an island in the Shetlands 66 km north-west of Fair Isle. The survey was

carried out by systematically quartering the breeding grounds and observing which pair of birds attacked or feigned injury in a particular area. The data of the numbers of breeding pairs are shown in Table 1 with theoretical frequencies given by a model of assortative mating analysed previously⁵. In the model, a proportion of the birds of each phenotype mate assortatively with others of similar phenotype; the remaining birds mate at random. Pale, intermediate and dark birds mate assortatively in the proportions α , β and γ . Female preferences generally determine assortative matings, but the model allows for both male and female preferences.

If the intermediates and darks in the data of Table 1 are lumped together, the analysis of χ^2 is similar to the analysis of the data published earlier. There is a significant overall deviation from random mating, but not in the Fair Isle data alone. This difference between the colonies is not itself significant. If the intermediates and darks are separated, however, the data of both Fair Isle and Foula show very significant deviations from random mating, but in different directions. The parameters α , β and γ of the general model of assortative mating have been fitted to the data by maximum likelihood. The effects on χ^2 of fitting the different parameters are shown in Table 2. Fitting the single parameter β to the Fair Isle data reduces the value of χ^2 to less than the degrees of freedom. χ^2 is further reduced, but not of course significantly, by fitting the additional parameter γ . Fitting the parameter α to the Foula data similarly reduces χ^2 to an insignificant value and fitting β reduces it still further. Our data are thus explained if 47% of intermediates mate assortatively on Fair Isle and 32% of pales mate assortatively on Foula.

These inferences are confirmed by the log likelihoods and support limits given in Table 3. Unless β is fitted to the Fair Isle data and α to the Foula data, the log likelihoods are always more than two units of support below the maximum. The support limits show the precision of the maximum likelihood estimates of these parameters.

What might explain the differences we have found and what consequences may follow? O'Donald⁴ suggested that imprinting might be the explanation but found there was no evidence for imprinting in data on the matings of parents and the subsequent matings of their chicks. If imprinting were a cause of the females' mating preferences, it would produce neither the strong assortative mating of intermediates on Fair Isle, nor the difference between the colonies. An alternative explanation would be a genetic difference between the colonies in mating behaviour. This might have arisen by chance as a 'founder effect', or evolved by selection if pairs differ in breeding success on the islands. Birds fledged in one colony sometimes breed in other colonies, however; and if this migration is random, it must reduce any genetic differences between colonies. The variation in assortative mating thus presents a remarkable evolutionary problem for future researches.

Assortative mating has the effect of reducing heterozygosity.

Table 2 Estimation of the parameters α , β and γ in the models of assortative mating for the Arctic skuas of Fair Isle and Foula

Parameters estimated	Arctic skuas of Fair Isle				Arctic skuas of Foula			
	ML estimates of parameters		χ^2	d.f.	ML estimates of parameters		χ^2	d.f.
None	—	—	9.950	3	—	—	11.556	3
α	0.102	—	9.110	2	0.316	—	1.923	2
β	0.474	—	1.385	2	0.276	—	6.802	2
γ	0.237	—	5.797	2	0.015	—	11.501	2
α and β	0.0	0.475	1.385	1	0.288	0.180	0.538	1
α and γ	0.089	0.233	5.485	1	0.315	0.0	1.923	1
β and γ	0.437	0.165	0.111	1	0.275	0.0	6.801	1

The maximum likelihood (ML) estimates were found by calculating the log likelihood (base e) for a given set of values of the parameters. Random variations in the values were then introduced until a higher log likelihood was obtained. This process was repeated with smaller and smaller random variations until the maximum likelihood was reached. Explicit solutions of the equations for maximum likelihood can be obtained if in addition to the phenotypic frequencies only one parameter is to be estimated. The explicit solutions give the same values as those found by the random search.

Table 3 The log likelihoods and support limits of the estimated parameters

a, Arctic skuas of Fair Isle		Maximum log likelihood	
Parameters estimated		(log L_{max})	
β		-181.754	
β and γ		-181.141	
α, γ, α and γ		-183.625	
Support for β relative to log L_{max}		-181.754	
Units of support		Values of β	
		Upper limit	Lower limit
3 units		0.692	0.097
2 units		0.660	0.184
1 unit		0.613	0.283

b, Arctic skuas of Foula		Maximum log likelihood	
Parameters estimated			
α		-283.028	
α and β		-282.364	
β, γ, β and γ		-285.388	
Support for α relative to log L_{max}		-283.028	
Units of support		Values of α	
		Upper limit	Lower limit
3 units		0.523	0.069
2 units		0.488	0.117
1 unit		0.441	0.177

The levels of support are units of log likelihood below the maximum log likelihood. For example, for the Fair Isle skuas, the 2-unit support limits are the values of β for which $\log L = -183.754$, being two units of log likelihood below the maximum when the one parameter, β , is estimated. If variation is normally distributed, the 2-unit limits correspond to the limits $\pm 2\sigma$, where σ is the standard error of the estimate. The variation in these estimates, is however, not normal and the limits are not symmetrical, although the difference between the upper and lower 2-unit limits is approximately 4σ . The difference between these limits would be exactly 4σ if the variation were normal.

O'Donald⁵ showed that at equilibrium the frequency of the heterozygotes is given by the solution of the equation

$$[(1-\frac{1}{2}\beta)(\frac{1}{2}\alpha-\beta+\frac{1}{2}\gamma)-\frac{1}{2}(\alpha-\beta)(\gamma-\beta)]v^2+[(1-\frac{1}{2}\beta)(1-\alpha p-\gamma q)-q(\alpha-\beta)(1-\gamma)-p(\gamma-\beta)(1-\alpha)]v-2pq(1-\alpha)(1-\gamma)=0$$

where p and q are the gene frequencies and v is the frequency of the heterozygotes. To apply this formula to the Arctic skuas, we must assume that the intermediates are the heterozygotes⁶. There is in fact a continuous range of variation from intermediates to completely dark birds. In our present work we have classified as intermediate any bird with at least some paler feathers on the nape. Dark birds seem to be homozygous by this classification. Using our estimates of the parameters, we predict that the frequencies of heterozygotes at equilibrium should be 0.4748 on Fair Isle and 0.4563 on Foula, compared with the actual frequencies of 0.5905 and 0.4606 respectively. The predicted frequency is very close to the actual frequency on Foula, but very significantly different from it on Fair Isle. Indeed, on Fair Isle, there is a significant excess of intermediates over the numbers of heterozygotes expected by random mating. This may indicate that natural selection favours heterozygotes on Fair Isle or that differential migration has occurred and equilibrium has not yet been reached.

Assortative mating differs from sexual selection only to the extent that the mating preferences are restricted to individuals of the same phenotype as that which is preferred. Assortative mating and sexual selection will have similar selective effects as a result of female mating preferences: the preferred males will gain an advantage because they will mate earlier in the breeding season. The preferences have been estimated from the Fair Isle data of breeding dates of pale, intermediate and dark males³. In the model with the highest likelihood, 18% of the females prefer dark males and 29% prefer intermediate males. Using our present data, we have estimated that 47% of the matings of intermediates are assortative. These matings are probably caused by the preference of intermediate females

for intermediate males. The 47% of these females represents 29% of all females—almost exactly the estimate of all females who prefer intermediates.

Intermediate females breed on about the same average date as intermediate males. This is to be expected as a result of the assortative mating. The similar breeding dates of the other females show that the preference for dark males, which are the first to breed, is distributed at random among the female phenotypes, giving rise to sexual selection for the darks without assortative mating.

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Cascading speciation

CENTRAL to the theory of allopatric speciation is the idea that geographic separation of populations will sufficiently restrict gene flow between them to make possible their genetic divergence¹. Wright² has shown that, at equilibrium, the arrival of one migrant on average every other generation is sufficient to keep a population's genetic composition similar to the source of migrants—at least when selection is not too strong. Wright's result has been extended to one- and two-dimensional arrays by Maruyama³.

There may be some difficulty in describing the usual course of the speciation process in the light of these theoretical results. If one or several dispersing individuals from an established population can reach some location and start a new population, then what is to prevent others arriving from the same source and preventing local differentiation? One possible answer is that speciation depends entirely on infrequent and accidental events. For example, small and large scale geological changes can completely prevent gene flow that was once possible or the 'founder effect'¹ could produce rapid enough genetic changes in a newly-founded population that some degree of reproductive isolation could evolve before many more migrants arrive. It is also possible that many species evolve from migrants which have gone sufficiently far from their parent colony that the arrival rate of other migrants from the same colony is far below Wright's limit.

Another mechanism for species formation and one which does not require accidental events is disruptive selection resulting from the ecological conditions in different microhabitats. Experiments^{4,5} have shown that reproductive isolation can sometimes evolve in the presence of gene flow and strong disruptive selection, and theoretical models⁶ reinforce that conclusion. Disruptive selection, however, cannot account for the abundance of sibling species or closely related species which are similar in geographic distribution and ecological requirements¹.

All these factors have been important in evolution, and it is possible that they are sufficient to account for the known rates

of speciation. I propose, however, that if the non-equilibrium properties of genetic systems are considered, then the existence of accidental processes or disruptive selection is not necessary for all cases of speciation. In particular, there is often no need to assume that there is some mechanism which 'shuts the door' on future migrants so that speciation may occur.

My argument follows from well known theoretical results. For a diploid species, consider a population of size N which receives m migrant individuals from a second population, and consider a locus segregating for two alleles A and a . Suppose that A is fixed in the second population and a in the first. Then in each generation in which a migrant arrives at the first population, the probability of ultimate fixation (u) of either of the arriving alleles is

$$u = [1 - \exp(-4s)] / [1 - \exp(-4Ns)] \quad (1)$$

where s is the selective advantage of A over a (assuming no dominance and independence of the alleles⁷). For $Ns \ll 1$, u is approximately $1/N$ and for $Ns \gg 1$, approximately $4s$. If we assume that each migrant is an independent trial, then the expected time until the arrival of the A allele which will ultimately be fixed is $1/mu$. The expected time until fixation of the successful allele must be added to its expected arrival time⁸. Thus, there is a time lag in the response of a population to gene flow. In this example, if $m = 1$ and $s = 0.01$ the time lag until fixation begins (which is a minimum estimate of its importance) is 25 generations. With $s = 0.001$, the lag increases to 250 generations.

If instead we assume A is increasing deterministically in the second population according to

$$p(t) = \left[1 + \frac{(1-p_0)}{p_0} \exp(-st) \right] \quad (2)$$

where p_0 is its initial frequency⁴, then the average number of A alleles carried by each migrant is $2p(t)$. The probability of ultimate fixation is given approximately by equation (1) so the expected time until fixation of A begins in the first population is

$$\begin{aligned} & \int_0^\infty tump(t) \exp\left[-\int_0^t ump(t') dt'\right] dt \\ &= \int_0^\infty \exp\left[-\int_0^t ump(t') dt'\right] dt \end{aligned} \quad (3)$$

where the second expression is obtained by integrating by parts. With $m = 1$, $p_0 = 0.001$ and $s = 0.01$, the time lag is 420 generations and with $s = 0.001$, 4,200 generations. Before A is established another allele serving the same purpose could arise and become fixed. In this way genetic differences could accumulate.

With only two populations, each of which is relatively permanent, the time lag effect may not lead to the evolution of genetic differences, as it requires that new alleles with similar functions arise within a relatively short time. If there were a large number of populations in an array, however, then the time lag would increase roughly linearly with the number of steps separating two populations, and consequently could be significant for populations at opposite ends of the array. The wave of advance of an advantageous allele, which was modelled by Fisher⁹, would be considerably slowed.

The time-lag effect could also be important when the expected time until extinction of a population is of the same order of magnitude as the time lag. If there were a collection of populations, each of which had some probability of providing the propagules to found new colonies and each of which went extinct at a certain rate, then genetic differences could develop

in different populations as a result of independent mutations in each. The time lag effect would prevent swamping by gene flow between the populations. Such a process could account for the genetic differences between populations of *Mus musculus*¹⁰ taken from a single barn. Those differences would be difficult to explain using an equilibrium theory.

The accumulation of small genetic differences by the combination of the time lag effect and the extinction and colonisation of local populations can be thought of as a cascading process in which the new populations which are founded are likely to be slightly different from the average of the collection of populations. The differences can result both from selection and genetic drift. The accumulation of differences can lead to speciation. I will call this process 'cascading speciation' to distinguish it from speciation processes in which populations are approximately in equilibrium. Cascading speciation skill depends on accidental events, namely extinction and recolonisation, but ecological studies have shown that extinctions and colonisations are relatively frequent events in many species¹¹, and can take place on the same time scale as the time lag associated with gene flow.

If cascading speciation has been an important process, then one consequence is that speciation rates within taxonomic groups with many fugitive species¹², which presumably have higher extinction rates and greater dispersal ability, would be higher. If equilibrium processes have been more important, then in such groups speciation rates would be lower because of the higher level of gene flow resulting from the greater dispersal tendencies. One possible example of this is the *Drosophila* of Hawaii¹³ which contains a large number of species and for which there is assumed to be a high rate of extinction of local populations¹⁴. Carson¹⁴, in discussing the Hawaiian *Drosophila*, has emphasised the importance of inbreeding in small local populations which would enhance the founder effect and the role of genetic drift; but that does not change the limit on gene flow imposed by the equilibrium theory, which is independent of local effective population size.

In conclusion, I should emphasise that my point is quantitative, not qualitative. Geographical isolation is still necessary for speciation, but if the extinction of local populations is considered, then the degree of isolation necessary is not as great as is often assumed.

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Isolation by distance in *Liatris cylindracea*

SEVERAL factors may cause genetic correlation—that is a genetic relationship between individuals within a population—including small population size, self-fertilisation, positive assortative mating or isolation by distance. Isolation by distance occurs when gene dispersal is limited so that distant populations in a series or remote areas within a population become genetically differentiated. Under an isolation-by-distance model of genetic differentiation, spatial distance and genetic correlation between individuals or populations are expected to be negatively related, that is, the greater the distance the smaller the genetic correlation. I wish to present evidence for isolation by distance in the plant, *Liatris cylindracea* Michx. (Compositae), obtained by a genetic distance analysis of gene frequencies at 27 allozyme loci. This analysis revealed genetic differentiation across very small distances and demonstrated that a restriction of gene flow has a profound effect on the spatial distribution of alleles within a population.

Plant species are prime candidates for the study of isolation by distance since they are immobile and gene dispersal in both the pollen and seed phases is often quite restricted. Indeed the earliest study of isolation by distance was on the annual herb *Linanthus parryae*, which shows genetic differentiation for flower colour¹. *Liatris cylindracea*, a prairie gayfeather, is a perennial, obligately outbreeding herb which grows in undisturbed prairies throughout the mid-west of the United States. A single, dense population² on a hillside in Zion, Illinois was divided into a grid 18×33 with 3 m² quadrats. Up to 60 plants were collected from each quadrat; the collection totalled 2,258 individuals. Starch gel electrophoresis was performed on the plants for 12 different enzyme systems, encoding 27 genetic loci. Gene frequencies were calculated on a per quadrat basis.

Evidence for isolation by distance was provided by analysis of genetic distance within the Zion population. Genetic distance is an index for measuring the accumulated number of gene differences per locus between groups of organisms. Of several available measures of genetic distance I used Nei's because it uses allozyme gene frequency data and, most importantly for a study of isolation by distance, it is related directly to Malecot's coefficient of kinship, ϕ . Nei's genetic distance is defined as:

$$D = -\log_e(J_{xy}/\sqrt{(J_x J_y)})$$

where J_x , J_y , and J_{xy} are the arithmetic means of the probabilities of two randomly chosen genes being identical in populations x , y , and x and y . Genetic distance is expressed as codon differences per locus.

Genetic distance in the *L. cylindracea* population was calculated for 103 quadrat pairs which had data from all 27

allozyme loci. Measurements of genetic distance between sample quadrats showed considerable variation. The mean genetic distance averaged from all of the distances computed is 0.0396 ± 0.004 codon differences per locus. Genetic distance between quadrat pairs ranged from 0.0061 to 0.1156.

Considerable local differentiation within the population is indicated by the genetic distance measures. If genetic differentiation is the result of restricted migration, genetic distance would be expected to be related to spatial distance. The average genetic distance measured between sample quadrats is plotted against spatial distance in Fig. 1. As spatial distance increases between sample quadrats, the genetic distance increases proportionately. This linear relationship of spatial and genetic distance suggests that isolation by distance has occurred in the Zion population.

These results are consistent with the population biology of *L. cylindracea*. Both phases of gene dispersal, pollen and seed, are very restricted³. Pollen is transferred mainly by bees; field observations on *L. cylindracea* indicate that most moves by pollinators are to the nearest neighbour. Likewise seed dispersal, although by wind, is limited. This restricted gene dispersal is manifested in the spatial variation of gene frequencies within the population. Statistically significant gene frequency differences of as much as 0.2 existed between neighbouring quadrats. Genetic variation at a single locus, however, generally does not correlate to environmental parameters within the population². Genetic divergence over distance appears to be a function of random gene frequency fluctuations. Only when all loci are considered together does the pattern of genetic variation in the Zion population emerge; genetic distance is positively related to spatial distance. This relationship is as predicted for a population in which gene dispersal is limited and genetic differentiation results from isolation by distance.

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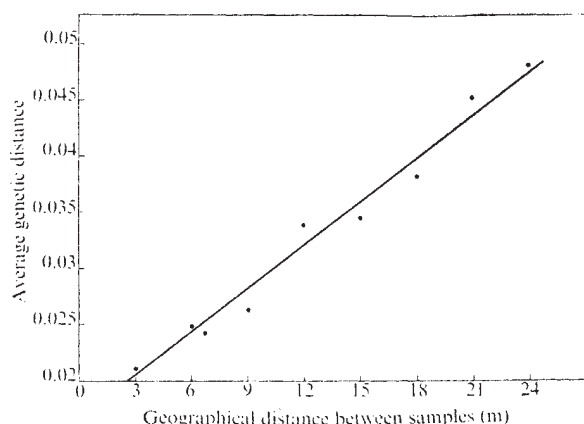


Fig. 1 Genetic distance in *L. cylindracea*. The genetic distance plotted is the average of all quadrat pairs for a given spatial distance. Geographical distance is the distance (m) between sample quadrats.

Implications of ethylene production by bacteria for biological balance of soil

ETHYLENE in soil causes fungistasis¹, affects the growth of bacteria, actinomycetes and nematodes (unpublished data) and consequently must influence many soil biological processes. *Mucor hiemalis* has been proposed as a major producer of ethylene in soil² although earlier work suggested that facultative anaerobes may be involved³. Our studies show clearly that the main production of ethylene in soil is by spore-forming bacteria in anaerobic microsites. These bacteria seem to be an integral part of a self-regulating cycle in soil controlling microbial activity and with far-reaching implications for the biological balance of soil.

A high organic matter, basaltic soil (Ashburner¹), which produces high levels of ethylene, was treated moist for 30 min with aerated steam at 60°, 82° and 100° C to simplify progressively the soil microflora⁴. Samples of treated soil were compared for ethylene production¹ with untreated and autoclaved (60 min at 121° C) samples. Untreated and treated soil samples were dried aseptically at 37° C for 24 h, passed through a 0.2-cm mesh sieve, placed as 5-g lots in four replicate sterile glass vials (14 ml capacity), wetted to 40% water (–0.5 bar water potential) with sterile distilled water, sealed with rubber septa and incubated at 25° C. This is our standardised assess-

ment method. Production of ethylene was reduced significantly only in soil heated above 82° C (Fig. 1). Small quantities of ethylene were detected following treatment at 100° C, but virtually none formed in autoclaved soil. Spore-forming bacteria (both aerobic and anaerobic) were the only significant survivors in soil treated at 60° C and above; some survived the treatment at 100° C, but none survived 121° C. Similar results were obtained with several other soils after treatment with aerated steam. In another test, soil slurries in tubes were heated at 80° C for 1 h in a water bath and then compared for ability to produce ethylene with non-treated slurries when incubated at 25° C. The treatment enhanced ethylene production, especially initially, probably because of heat activation of spores⁵. These results rule out *Mucor hiemalis* as a major producer of ethylene in soil² because it is killed by aerated steam at 60° C or above⁴.

In another series of experiments, Ashburner soil was wetted to different water potentials; ethylene was produced at -5 bar and wetter, but only slightly or not at all at -15 bar or drier. Soil bacteria, but not actinomycetes or fungi, are limited by water potentials below -15 bar⁶.

Production of ethylene under aerobic as compared with anaerobic conditions was studied with Ashburner soil wetted to 40% water and incubated at 25° C in the vials under atmospheres of air, nitrogen, argon or hydrogen. The atmospheres in vials of dry soil were evacuated twice and replaced each time with the desired atmosphere. Oxygen-free water was then added by syringe to the soil surface and allowed to move as a wetting front to the bottom of each vial. In all tests, ethylene production was greatest in the absence of oxygen (Fig. 2). A similar result was obtained with a cultivated garden soil (Penrith¹) which normally produced little ethylene. Additionally, ethylene production under a nitrogen atmosphere in Ashburner soil was prevented, or nearly so, by a water potential of -15 bar, confirming the role of anaerobic bacteria.

Amendment of soils with different forms of nitrogen markedly affected ethylene production. Nitrate always delayed production in proportion to the amount added whereas ammonium stimulated production, or had no effect, depending on the soil used. Ethylene production was best in soils leached free of nitrate but if such soils were reamended with nitrate at 20-200 p.p.m. N, production started only after a period of denitrification, or not at all. Nitrate acts as a terminal electron acceptor in the absence of oxygen and can poise the redox potential of soil sufficiently to prevent activity of strict anaerobes^{7,8}. This seems to explain how the presence of nitrate prevents ethylene production and indicates that production occurs only under highly reduced conditions in soil.

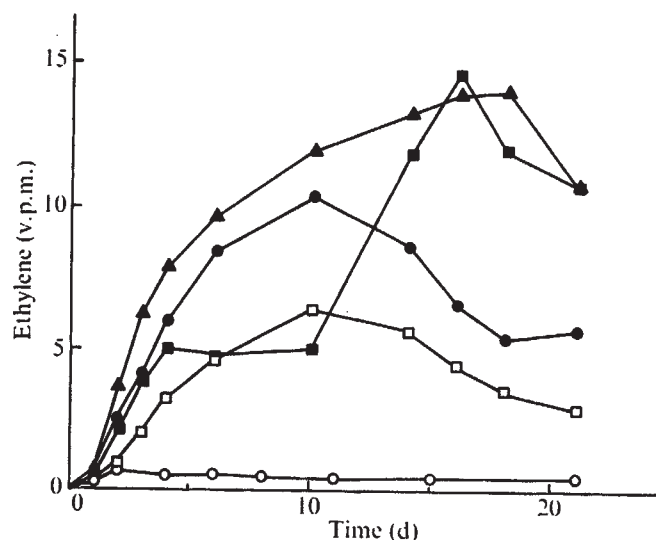
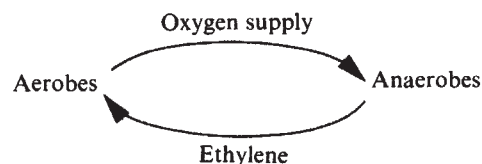


Fig. 1 Ethylene production by Ashburner soil following heat treatment. ●, Untreated; ▲, aerated steam for 30 min at 60° C; ■, 82° C; □, 100° C; ○, and autoclaved for 60 min at 121° C.

Although ethylene production was best in oxygen-free atmospheres, it was consistently formed, sometimes at high levels, in soil incubated under air and as dry as -5 bar water potential. It was also detected *in situ* at adjacent sites in a soil under mown grass (about 0.5-6.0 p.p.m.) and cultivated (about 0.03-0.5 p.p.m.), including when the soil was considerably drier than field capacity and hence probably well-aerated.

Any apparent conflict in the presence of ethylene in soil under the conditions described and its anaerobic requirements for production, are explained by the occurrence of anaerobic microsites in even well-drained field soil. These anaerobic microsites are continually formed in soil as a result of aerobic microorganisms utilising oxygen especially near organic matter⁹. Anaerobes will proliferate in these microsites and produce ethylene which diffuses through the soil. Sensitive species of aerobic microorganisms will be inactivated first, but eventually if levels become high enough, most, if not all, aerobic microbial growth probably will be arrested by the ethylene. The system then becomes self-regulatory because as oxygen demand falls, oxygen will diffuse back into the microsites and prevent anaerobic ethylene production; this will permit resumption of aerobic growth. We also have evidence that the anaerobes depend, at least in part, on aerobes for production of substrate for ethylene formation. This cycle may be summarised:



Verification of such a cycle comes from numerous observations made during the glass vial tests on ethylene production in soil. Prolific hyphal growth was observed through the glass vials under a dissecting microscope on the moist soil crumbs when ethylene was low but not when ethylene was high. In tests allowed to continue for over 80 d, ethylene levels reached a peak, dropped, then eventually rose again in cyclic fashion; fungal hyphae appeared, disappeared (lysed) and reappeared in response to the fluctuations. Actinomycetes behaved similarly except that colonies became evident only at intermediate levels of ethylene and after most fungal hyphae had lysed. At high levels of ethylene these colonies also disappeared.

Large inputs of nutrient temporarily override the suppressive effects of ethylene possibly in much the same way fungistasis is annulled by nutrients¹. Without this means of adjustment, decomposition of organic matter would be slowed, with consequent problems of accumulation in soil.

The concept of an oxygen-ethylene cycle assigns an important microbiological role to the ubiquitous spore-forming anaerobes of soil; by producing ethylene, they prevent microbiologically active soils from becoming predominantly anaerobic. This ensures that the more efficient aerobic respiration dominates the soil system. Anaerobic production of ethylene also explains why ethylene producers do not dominate a soil—anaerobes are prisoners of their microsites.

In our studies, bare cultivated soil has always produced less ethylene than undisturbed or cropped soil regardless of whether measured *in situ* or removed, dried, sieved and tested in the standardised vial test in the laboratory. This has important implications for agriculture because these results indicate that the oxygen-ethylene cycle is in balance in soil under undisturbed ecosystems such as grasslands and forests, where organic matter and plant nutrients are slowly recycled, nitrate nitrogen does not accumulate, and root diseases are rare. Conversion to agricultural usage destroys the balance because tillage aerates the soil, elevates the redox potential and eliminates anaerobic microsites. This increases organic matter breakdown

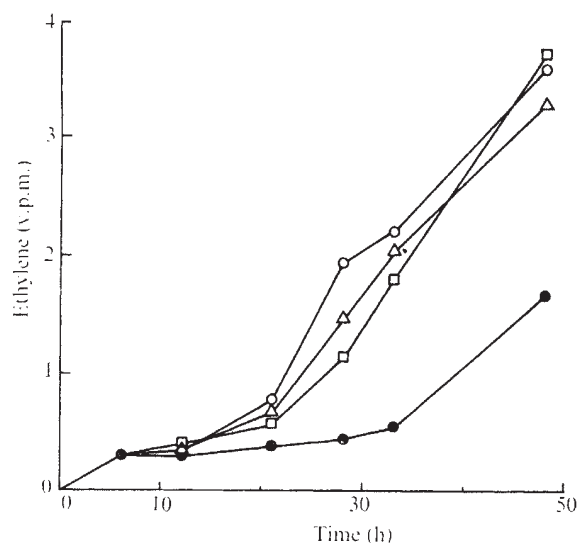


Fig. 2 Ethylene production by Ashburner soil under atmospheres of ●, air; ○, nitrogen; △, argon; □, and hydrogen.

and release of nitrogen. Plants are not present to use the ammonium nitrogen which is rapidly nitrified. Even if oxygen-free microsites redevelop, ethylene production will not resume until nitrate has been removed by plant roots of a subsequent crop, or less desirably, by leaching or denitrification. This seems to explain adequately why cultivation causes an inevitable decline in levels of organic matter, soil structure, and nitrogen availability and an increase in root diseases. Also, the presence of nitrate causes a loss of cations from soil¹⁰ and a paucity of reduced microsites which affects the availability of iron, manganese and phosphorus⁸.

Precise manipulation of the oxygen-ethylene cycle in soil offers a means of regulating energy turnover and availability of plant nutrients as well as improved control of soil-borne plant pathogens. These results call for a reappraisal of the value of nitrate in agriculture and give new justification for minimum tillage and the use of organic amendments in farming.

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Pigments responsible for ultraviolet patterns in flowers of *Oenothera* (Onagraceae)

MANY insect-pollinated flowers have nectar guides¹ which increase the foraging and pollination efficiency of their insect visitors². Some of these are visible only by their contrasting ultraviolet patterns, visible to insects but not to humans³. In such flowers, the nectar guides absorb ultraviolet light, and the compounds responsible were early suspected to be phenolics⁴. Recently, in *Rudbeckia hirta* L. (Asteraceae), a mixture of methylated flavonol glycosides has been shown to be responsible⁵. We report here that another class of flavonoids, chalcones, is responsible for the similar patterns in *Oenothera* (Fig. 1) and in three other genera of Onagraceae with flowers that appear uniformly yellow to humans.

Fresh petal material of *Oenothera hookeri* Torr. & Gray ssp. *venusta* (Bartlett) Munz was divided into ultraviolet-reflective and ultraviolet-absorptive portions. Because these areas cannot be distinguished visually, only small portions of the base and apex of the petal were used to minimise contamination. The material was extracted with methanol for several days to remove visible as well as ultraviolet-absorbing material. Extracts were analysed spectrally and chromatographically.

The absorption spectrum of the extract from the apical reflective portion of the petal had maxima at 460 nm, 435 nm and 415 nm (characteristic of carotenoids), a second maximum at 265 nm (probably non-flavonoid phenolic compounds) and a minimum between 310 nm and 370 nm (Fig. 2A). Extracts from the basal, absorptive portion of the petal had an additional strong maximum at 365 nm (Fig. 2A).

The two-dimensional paper chromatogram of the apical extract indicated that only carotenoid was present. The basal extract contained carotenoid plus an ultraviolet-absorbing pigment which was identified as the chalcone glucoside isosalipurposide⁶. The absorption spectrum of a methanol solution



Fig. 1 Fresh flower of *Oenothera grandis* in daylight (top) and in ultraviolet light (366 nm) with a Kodak Wratten 18A filter (bottom).

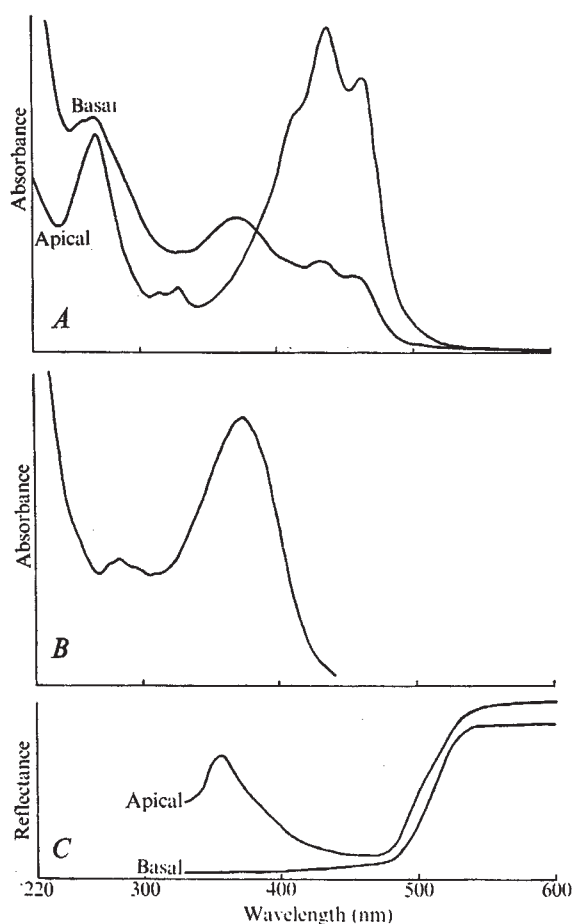


Fig. 2 A, Absorption spectra of methanol extracts of portions of petals from *Oenothera hookeri*. B, Absorption spectrum of a methanol solution of the chalcone, isosalipurposide. C, Reflectance spectra of portions of a petal of *O. hookeri*.

of this compound had a single absorption maximum at 365 nm (Fig. 2B). Thus the restricted distribution of the chalcone in the basal portion of the petals results in the observed differences in light reflection. The reflectance spectra of the two portions of the petal (Fig. 2C) also support this conclusion.

The internal anatomy of the petal suggests that the mesophyll is a diffusing and pigmented structure with transparent plates composed of epidermis on both surfaces⁷. Thus much of the light striking a petal is reflected unless it is absorbed by pigment molecules in the mesophyll. In the case of the basal portion of *Oenothera* petals, the presence of flavonoids absorbing in the near ultraviolet and carotenoids absorbing between 400 and 470 nm results in low reflection of wavelengths 330–480 nm (ultraviolet and blue light) with high reflection in the yellow region of the spectrum giving rise to yellow coloration for both humans and insects. The apical portion of the petal containing only carotenoid reflects ultraviolet as well as yellow light. Thus it appears yellow to humans but bee purple (a combination of ultraviolet and yellow light analogous to human purple which we distinguish when light from both ends of our spectral sensitivities, red and blue, are mixed) to an insect.

The observation of fluorescent patterns under ultraviolet light in pressed herbarium specimens matching the invisible ultraviolet patterns in fresh flowers⁸ fits this model of petal pigmentation. Flavonoids are stable while carotenoids are liable to autooxidation in air^{9,10}. The breakdown products of carotenoids presumably fluoresce under ultraviolet light, whereas the stable flavonoids continue to absorb. Flavonoids commonly appear as dark spots on paper chromatograms illuminated with ultraviolet light.

Other *Oenothera* species with yellow flowers which absorb

ultraviolet light were examined chromatographically. These represent not only the closely related subgenera *Oenothera* (*O. elata* HBK., *O. mayssilesii* Nutt. ex Torr. & Gray) and *Raimannia* (*O. drummondii* Hook., *O. grandis* (Britton) Smith, *O. rhombipetala* Nutt. ex Torr. & Gray) but also the more distantly related *Hartmannia* (*O. epilobiifolia* HBK., *O. seifrizii* Munz). Results were similar to those for *O. hookeri*, showing carotenoids present throughout the petal and the chalcone restricted to the absorptive areas. Another group of flavonoids, quercetin derivatives, was found in addition to the chalcone in the basal portions of the petals of all species, although not as abundantly in *O. grandis* and *O. rhombipetala*. Myricetin derivatives were also absent from petals of subgenera of *Oenothera* and *Raimannia* and present in those of *Hartmannia*, as reported for their leaves^{11,12}. Like the chalcone, myricetin derivatives have absorption maxima in the near ultraviolet.

With the results presented here, two classes of flavonoids, flavonols (in Asteraceae)⁵ and chalcones (in Onagraceae), have been found associated with absorption of ultraviolet light in nectar guides, indicating that these compounds together with anthocyanins and carotenoids are important in flower pigmentation. The chalcone isosalipurposide plays a role similar to that demonstrated here in *Oenothera* in other yellow-flowered Onagraceae belonging to the genera *Camissonia*, *Gaura* and *Ludwigia*⁶.

The involvement of flavonoids in these pigment systems important in plant-pollinator interactions suggests a role for the flavonoids which are ubiquitous in the leaves of angiosperms and contribute to absorption in the ultraviolet portion of the spectrum. Leaves reflect a low level of light with spectral quality such that insects perceive it as grey, a neutral colour³. This means that plants which depend on insect visitors for pollination can advertise their flowers as brightly coloured entities against a dull, neutral background and thus increase the efficiency of their pollinators.

Voucher specimens are deposited in the Dudley Herbarium, Stanford University (DS). This work was supported by grants from the National Institutes of Health and the United States National Science Foundation. We thank Howard Towner for the photographs.

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Tumbling in pigeons

THE spectacular hereditary trait of backward somersaulting by Tumbler pigeons has been known since 1600 AD¹ and was described by Darwin as “. . . one of the most remarkable

inherited habits or instincts ever recorded...². The potentially important physiological mechanism of tumbling is unknown, largely because the extreme rapidity of the somersaulting blurs visual observation of the component motions³⁻⁹. Filming at 2,000 frames per second the normal flight of Racing Homer pigeons and characteristic tumbling episodes by Parlor (non-flying) Tumbler pigeons, we observed the following. The body position of a pigeon just before and during tumbling is associated with abnormal dorsiflexion of the tail, which occurs within 15 ms after the pigeon is released. Tumbling is synchronised with apparently normal wing movements, propelling the pigeon in only a backward direction, at a rate of 8–10 somersaults per second.

Each filming session involved releasing a hand-held pigeon 0.3 m above a flat surface and activating the camera and high intensity lamps for a period of 1–2 s. Although we were mainly interested in the initial tumbling motions, 5–10 somersaults by each pigeon were filmed during this short time. The film was analysed frame-by-frame and at 16 frames s⁻¹. Sketches from representative frames with corresponding times (ms) are shown in Fig. 1.

Figure 1a shows that the normal pigeon raises the wings and extends the feet upon release and lands on the feet with wings forward. In addition, the head, body and tail remain in line

and parallel to the surface below during the descent. Frames 4–7 in Fig. 1a illustrate the initial flying motions, consisting of phasic wing beats accompanied by a forward shift of the body mass that result in flying less than 200 ms later. As in the descent, the head, body and tail remain in line as the pigeon ascends.

Pigeon fanciers and researchers alike use number of somersaults per unit time, duration of a tumbling episode and ease of provocation of tumbling to categorise Tumbler pigeons³⁻⁷. A severely affected bird will begin somersaulting with each mild provocation such as approaching the bird or snapping the fingers. Less severely affected birds sometimes simply run from the investigator and may somersault only in response to a loud hand clap or to dropping from a height.

The pigeon depicted in Fig. 1b is a severely affected Parlor Tumbler, while that in Fig. 1c represents a milder form. The severely affected Tumbler raises the wings and extends the feet upon release (frames 1 and 2) as does the normal pigeon (Fig. 1a) but the head and body of the Tumbler are not parallel to the surface below. Also the tail of the Tumbler forms an acute angle with the rest of the body. The tail elevation in the Tumbler occurs within 15 ms of release (not shown) and the tail remains in this abnormal position throughout the tumbling episode. The milder form (Fig. 1c) lands with feet and wings extended forward (frame 3) but neither wings nor feet

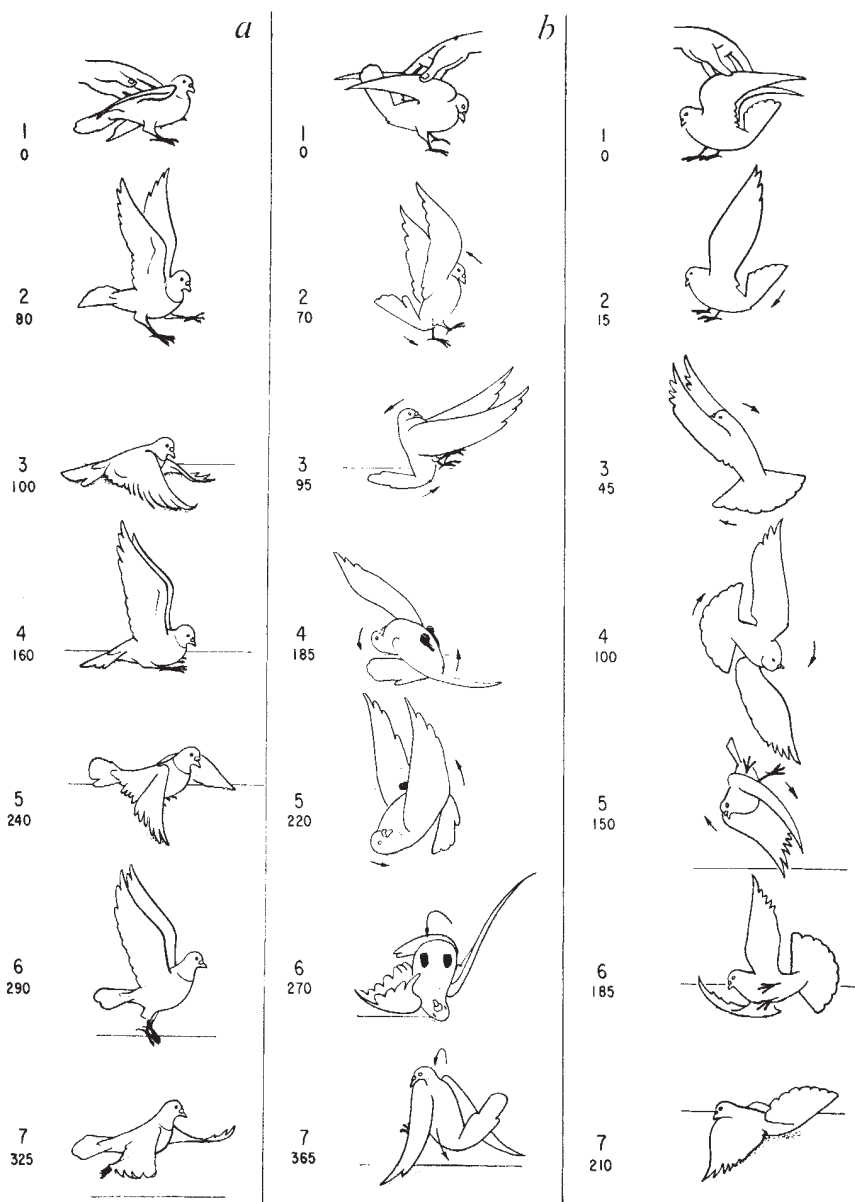


Fig. 1 Movements of normal and Parlor Tumbler pigeons in response to release 0.3 m above a flat surface. *a*, Normal pigeon; *b*, severely affected Parlor Tumbler pigeon somersaulting upon contact with surface; *c*, mildly affected Parlor Tumbler pigeon somersaulting in flight. Each pigeon was released at frame 1. Numbers 1–7 refer to successive frames. Time (ms) given below each frame number. Zero time was taken as the moment of first discernible downward movement.

contact the surface as in the normal pigeon. The abnormal landing appears to be related to the backward angular rotation (nose-up pitching) during descent. Both wings and tail may be involved in the rotatory movement, but we suggest that the tail is the main influence. The initiation of the somersault is seen in Fig. 1*b* (frame 3) where the pigeon has started to pitch as the wings move forward. Subsequent wing movements appear appropriate for flight, but the abnormal posture maintained during tumbling causes the wing beating to propel the pigeon backwards. A cycle of a single wing beat in the normal pigeon and severely affected Tumbler *b* lasts 100–125 ms; the head and body remain aligned in both, but the tail is elevated only in the Tumbler. Subsequent somersaults are stereotyped and occur at a rate of 8–10 per second with one wing beat per somersault.

Figure 1*b* also shows that movements other than pitching rotation occur during tumbling. These are rotation about the longitudinal body axis (rolling) and rotation about a dorso-ventral body axis (yawing) which cause the pigeon to land in a position different from that expected after a simple 360° pitching motion. Comparison of frames 3 and 7 in Fig. 1*b* illustrates the effect of these superimposed motions.

Figure 1*c* shows tumbling in flight. Wings and feet are again extended and the tail elevated within 15 ms after release (frame 2). After 45 ms descent the pitching motion has positioned the pigeon abnormally but head and body remain aligned (frame 3). The propulsive force for tumbling in flight, as on the ground, is apparently supplied by the wings. The first evidence of tumbling occurs in frame 3 as the forward wing stroke begins; 140 ms later the episode ends in an awkward landing as the cycle is completed (frame 6). Frames 4 and 5 illustrate the rolling and yawing motions, and their influence on the pigeon's ultimate position.

All the Tumbler pigeons we observed had apparently normal wing motions. The force was supplied by the forward wing stroke during which the feathers appeared tightly interlocked. During the back stroke the wing feathers separated and sliced through the air with little resistance. Furthermore, the position of the wings relative to the body was the same in normal and Tumbler pigeons. The feet did not seem to contribute to tumbling; their position varied from one somersault to the next and no pushing movements were observed. In fact, in some cases the feet seemed to decrease the tendency to tumble by exerting a dragging force opposite in direction to the characteristic pitching motion.

The stereotyped abnormality of the Tumbler pigeon possibly arises from the abnormal function of a sensory input, a central or feedback mechanism, or an aspect of the motor output.

The fact that the abnormal response occurs within 15 ms of the pigeon being released is consistent with neural transmission of a stereotyped movement. The inner ears of Tumbler pigeons are normal^{6,7}, the cerebella appear normal by light microscopy (R.K.E., and S.H.B., unpublished observation) and earlier experiments indicate that tumbling is not a form of epilepsy^{5,6}. In addition, we have recently reported that skeletal muscles of Tumbler pigeons are not myotonic^{8,9}. Experiments in progress suggest that tumbling is associated with an abnormality of the central nervous system. Irrespective of the mechanism of tumbling, a better understanding of motor control in animals with prominent basal nuclei might be gained from further studies of Tumbler pigeons.

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Changes in heroin self-administration by a rhesus monkey after morphine immunisation

We have investigated in one primate the effect of immunisation against an opiate on the self-administration of these drugs. Using the rhesus monkey, which will self-administer various drugs, including opiates and psychomotor stimulants¹, we have followed the response for heroin before and after immunisation against morphine. Results indicate that antibodies against morphine can block those effects of heroin on the central nervous system (CNS) that maintain self-administration behaviour.

Our procedure was based on existing knowledge of the immune response against opiates. Antibody against morphine-protein conjugates has been demonstrated to bind free opiate haptens²⁻⁵. Different antibody specificities have been described when the same immunising opiate was linked to the protein carrier through different positions on the chemical nucleus^{3,5-8}. Antiserum obtained after immunisation with morphine-6-hemisuccinyl-bovine serum albumin (M-6-HS-BSA), the conjugate we used, has highest and approximately equal affinity for morphine and heroin and progressively less for opioids of decreasing structural similarity⁸.

In vitro studies based on the observation that morphine inhibits electrically stimulated contractions of isolated guinea pig ileum longitudinal muscle have shown that anti-M-6-HS-BSA globulin can block a peripheral pharmacological effect of morphine^{9,10}. Concentrated antibody was found to reverse the inhibition of muscle contractions caused by 120 nM morphine in a manner apparently identical to that observed after addition of 10 nM of the narcotic antagonist naloxone¹¹. The drug self-administration model enables extension of these studies to CNS actions of opiates *in vivo*.

The apparatus used was a modification of that illustrated by Schuster and Johanson¹. A double lumen polyvinyl chloride catheter was implanted into the right internal jugular vein of a 5.6 kg rhesus monkey and passed subcutaneously to an exit point on the animal's back.

While self-administering drug, the monkey was housed in a wooden cubicle and minimally restrained by a stainless steel harness and attached hollow steel spring arm. The catheter extended from its exit on the monkey's back through the arm, out the back of the cubicle and through a peristaltic infusion pump to connect with a drip bag reservoir of drug solution or saline. The cubicle was sound-attenuated and approximately 75 cm × 75 cm × 90 cm. The front door held an observation window, ventilation fan and two boxes, each with a lever and stimulus light. This allowed separate levers and stimulus lights to be associated with heroin and cocaine. A third stimulus light was located high on a side wall.

The monkey was trained to self-administer heroin and cocaine in aqueous solutions of 0.9% NaCl on a fixed ratio ten schedule (ten presses of the correct lever required for delivery of one infusion). Cocaine, which is not significantly bound by anti-

M-6-HS-BSA, was used to detect nonspecific changes in drug taking behaviour after immunisation. Such changes could conceivably result from toxic effects of the immunising preparation or from the lack of opportunity to self-administer drug during immunisation, although trained animals after periods of several weeks without access to drugs will readily resume self-administration (unpublished results of C.R.S.). Only one of the two drugs was available each day for a 2-h session, and the sequence of drug availability was varied. The appearance of a stimulus light overhead and another on the appropriate lever signalled the start of each drug session. During all infusions the lever light was turned off and the colour of the overhead light changed. Stimulus lights were controlled and lever presses and infusions taken were recorded by electromechanical programming equipment located in an adjoining room.

Infusion volume was 0.2 ml kg^{-1} delivered at approximately 6 ml min^{-1} . The heroin dose was $6 \text{ µg per kg per infusion}$, chosen to maintain self-administration behaviour without producing physical dependence that would complicate the two-drug baseline. The number of infusions taken per session for each drug was approximately equated by adjusting the cocaine dose, resulting in $100 \text{ µg per kg per infusion of cocaine}$.

After heroin and cocaine intake had stabilised, substitution of normal saline demonstrated that the self-administration of heroin and cocaine were independent of each other and that the monkey could detect the absence of either drug in spite of the presence of the usual stimuli associated with drug reinforcement. Two such heroin extinctions and one cocaine extinction observed before beginning immunisation are shown in Fig. 1. The first extinction for each drug showed a rapid drop to zero infusions self-administered per session. The second heroin extinction showed a more typical pattern, an initial increase in responding and number of infusions taken, followed by a drop to an infusion total per session well below the drug baseline but above zero¹². When drug solution was reintroduced in each case there was a prompt return to self-administration behaviour. During the saline infusions, intake of the unsubstituted drug varied only within the usual baseline fluctuations. The stability of heroin intake per session throughout this 2-month period also indicates that tolerance was not a significant factor regulating self-administration.

Drug sessions were discontinued and the monkey was transferred to a metal grid cage. He was then immunised subcutaneously with $5 \text{ mg M-6-HS-BSA conjugate}$ in complete

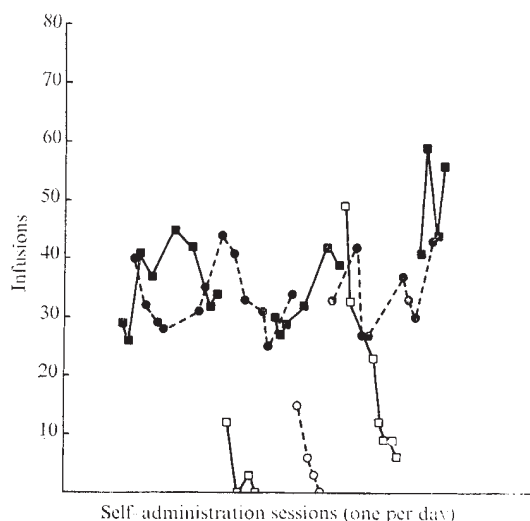


Fig. 1 Effect of substitution of saline for drug solutions on self-administration by a rhesus monkey. Total infusions taken per 2-h session are shown. ■, Heroin $6 \text{ µg per kg per infusion}$; □, saline infusions accompanied by heroin-associated discriminative stimuli; ●, cocaine $100 \text{ µg per kg per infusion}$; ○, saline infusions with cocaine-associated discriminative stimuli.

Freund's adjuvant. A second 5-mg injection was given 28 d later. Every 2 weeks thereafter 10 mg of the conjugate in incomplete Freund's adjuvant was given. After 20 weeks the serum morphine-binding capacity at $100 \text{ pmol ml}^{-1} \text{ }^{14}\text{C-morphine}$ added was $77,550 \text{ pmol ml}^{-1}$ undiluted antiserum⁸. Radio-immuno-electrophoresis demonstrated that the morphine was specifically bound to the IgG fraction of serum from the monkey. (We have also found high levels of morphine binding in antisera from two other monkeys immunised with M-6-HS-BSA.)

A catheter was implanted in the left internal jugular vein and the animal, now weighing 6.6 kg , was returned to drug self-administration. The monkey resumed his former baseline level of cocaine intake but failed to respond for heroin with a frequency significantly above that for saline (Fig. 2). The heroin dose was then successively doubled, allowing a minimum of three heroin sessions per dose. Self-administration was reinitiated at $100 \text{ µg per kg per infusion}$ with an average of 16 infusions taken per 2-h session. This is approximately 16 times greater than the original reinforcing dose per infusion and a concentration too depressant in the non-immunised animal to maintain self-administration (R. L. Balster, personal communication). A saline substitution extinction of lever pressing for cocaine was interposed at this time and as before had no appreciable effect on heroin self-administration. The

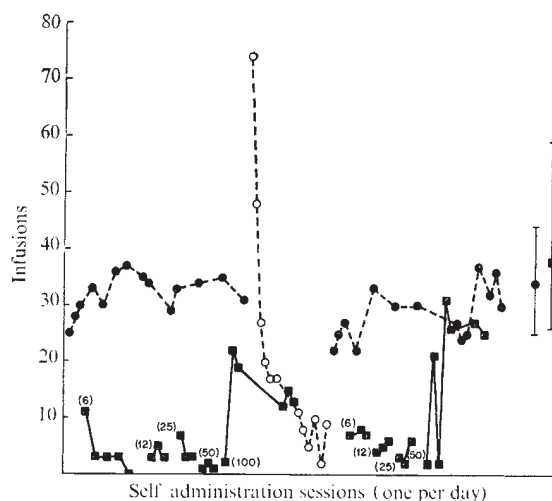


Fig. 2 Effect of immunisation on drug self-administration by a rhesus monkey, and effect of substitution of saline for cocaine after immunisation. Total infusions taken per 2-h session are shown. ■, Heroin infusions and heroin doses in $\text{µg per kg per infusion}$ shown in parentheses; ●, cocaine $100 \text{ µg per kg per infusion}$; ○, saline infusions with cocaine-associated discriminative stimuli. Mean and range of drug infusions per 2-h session before immunisation are shown.

animal was then returned to the 6 µg kg^{-1} heroin dose and the number of infusions taken per session again markedly decreased. In a second ascending series of heroin doses, self-administration was reinstated at $50 \text{ µg per kg per infusion}$ with an average of 26 infusions taken per 2-h session (Fig. 2). The increased number of infusions taken by the monkey the second time drug self-administration was reinitiated compensated for the reduced dose at which the reinitiation occurred. As Fig. 3 shows, when heroin self-administration was maintained after immunisation the monkey received approximately $1\text{--}2 \text{ mg per kg per 2-h session}$, an amount of heroin about 10 times greater than that necessary to maintain self-administration before immunisation.

During these studies the concentration of antibody decreased. As Fig. 3 shows, serum obtained at least 18 h after the heroin sessions showed a decrease in capacity to bind $^{14}\text{C-morphine}$ from $77,550 \text{ pmol ml}^{-1}$ undiluted serum to $7,900 \text{ pmol ml}^{-1}$, approximately 10% of its former level. This decline in antibody available to bind incoming heroin may account for the reinitiation of heroin self-administration at the 50 µg per kg per

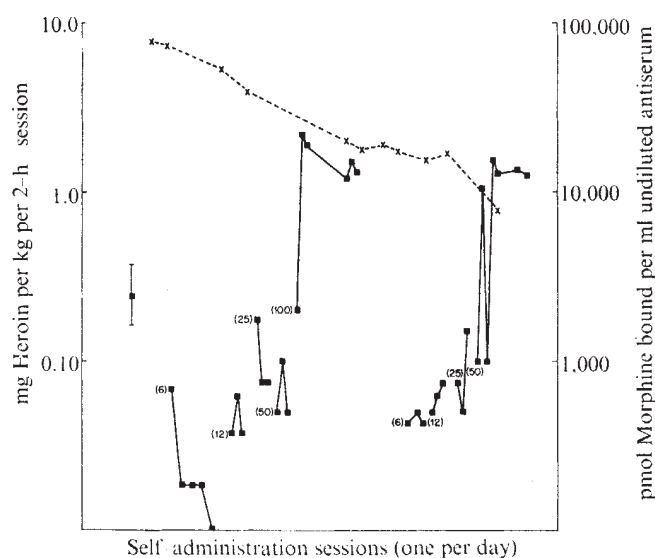


Fig. 3 Heroin intake and serum morphine-binding capacity after immunisation. ■, Heroin intake. Heroin doses in μg per kg per infusion are shown in parentheses. ×, Morphine binding capacity. Mean and range of heroin intake before immunisation are shown.

infusion dose in the second series of ascending heroin doses, although a learning effect cannot be ruled out. This decline in antibody concentration also shows that as in other immune reactions opiate antigen is non-immunogenic when bound by antibody in the presence of antibody excess¹³.

We next studied the time course of the heroin-antibody interaction by giving the monkey a single intravenous infusion of 1.0 mg kg^{-1} over 10 min and obtaining frequent serum samples during the next 2 d. The results summarised in Fig. 4 show that the antibody concentration decreased sharply 6 min after heroin infusion. By 3 h it had risen again to approximately three-quarters of the starting concentration and there was little change during the next 48 h. The heterogeneity of antigen-binding we observed is consistent with an earlier finding of both high and very low affinity antibody in M-6-HS-BSA-immunised rabbits¹⁴.

Cerebrospinal fluid (CSF) was obtained by lumbar puncture to learn whether the antibody blockade of heroin extended into the CNS. The CSF bound $600 \text{ pmol morphine ml}^{-1}$ undiluted fluid. The capacity of the serum to bind morphine, determined at the same time, was $10,899 \text{ pmol ml}^{-1}$, suggesting that most heroin binding takes place in the peripheral circulation.

The monkey was then allowed to self-administer only cocaine for 2 weeks, during which two booster immunisations were given. Four days after the second booster the monkey stopped

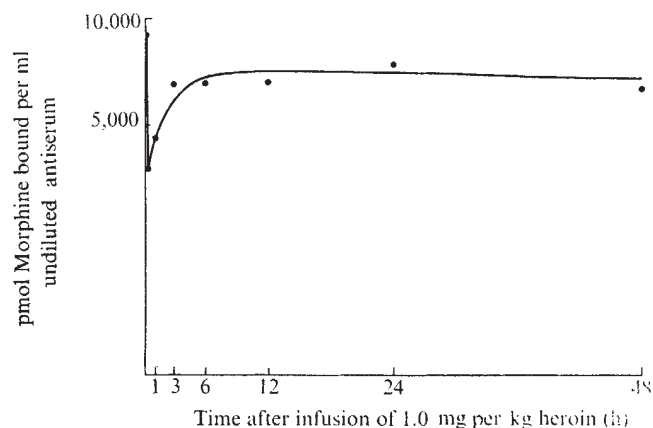


Fig. 4 Serum morphine-binding capacity after intravenous infusion of 1.0 mg kg^{-1} heroin.

self-administering cocaine. This marked the onset of a febrile illness which ended in the monkey's death 1.5 months later. Autopsy revealed widespread intranuclear inclusions in endothelial tissue supporting a diagnosis of disseminated viral infection. The kidneys showed hypercellular glomeruli with herpesvirus-like inclusions. Electron microscopy revealed intact basement membranes without areas of high electron density. These findings suggest that the monkey's death was not directly related to immunisation with M-6-HS-BSA.

Our results indicate that rhesus monkeys can be induced to produce antibody against opiates. The anti-opiate IgG is present in both serum and CSF. Blockade of CNS actions of heroin by this antibody can be demonstrated. Specifically, those actions of heroin that reinforce its self-administration in the non-dependent rhesus monkey can be blocked. This blockade has been shown to be dose dependent, and it can be overcome by high doses of drug.

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β Alanine and cuticle maturation in *Drosophila*

THE sclerotisation and tanning of insect cuticles is generally thought to result from a crosslinking of the cuticular proteins by quinonoid derivatives of tyrosine^{1,2}. Little consideration has been given to the fact that β alanine is a constituent of the cuticles of many insects^{3,4}, although it is required for normal tanning of the pupal case of several insects. Failure to utilise or produce the compound in *Drosophila melanogaster* results in an untanned pupal case⁵⁻⁷, whereas in *Musca domestica*, *Bombyx mori* and *Drosophila virilis*, failure to incorporate β alanine results in abnormally black cases⁷. In this work, we suggest an explanation for these observations and provide evidence that β alanine also plays a role in the tanning of adult fruit-flies.

Microinjection experiments were carried out as described elsewhere⁶. About 5 nmol of a sterile solution of β alanine (0.1 M) in insect Ringer⁸ were injected into the abdomens of pharate adults 0–24 h before eclosion. The microinjection of β alanine into pharate adults of the mutant *black* produced a striking permanent phenocopy of the normal body colour. Genotypically *black* organisms injected with insect Ringer developed the typical mutant body colour.

Recently we reported that substantial amounts of dopamine accumulate prior to eclosion in *D. melanogaster*⁹. Oxidation of this dopamine to indole-5,6-quinone would be expected when O_2 becomes available after eclosion, and melanisation would ensue if the indole-5,6-quinone molecules polymerised¹⁰. Because melanin deposition over a large part of the adult cuticle of *D. melanogaster* does not normally occur, we postulated that extensive polymerisation was prevented by β alanine. On this basis, the excessive pigmentation in the mutant *black* can be accounted for in terms of its failure to produce β alanine⁶. This contention is supported by our observation here that the injection of β alanine into *black* before eclosion prevents the mutant adult body colour from developing.

A result analogous to that obtained here for *black* adults has been reported for the black puparium (*bp*) mutant of *Musca domestica*⁷. Feeding larvae of this mutant on food supplemented with β alanine causes a normal (brown) puparium to form. We suggest that in those insects where the potential for abnormal melanisation at pupariation or eclosion exists (as demonstrated by the existence of mutants with excessively melanised cuticles), indole-5,6-quinone in conjunction with β alanine is required for normal tanning. Whether or not indole-5,6-quinone is involved in cuticle maturation at a particular stage in any insect would depend on the extent to which dopamine accumulates at this stage. In *D. melanogaster* the amount of dopamine present at pupariation is very low⁹, which is consistent with the fact that no mutant of this genus having a dark pupal case is known.

This model readily provides a possible mechanism for the extensive melanisation which does occur in the stripes along the posterior margin of the tergites of wild type *D. melanogaster*. The obvious correlation between the location of the oenocytes and the pattern of melanin deposition in the abdominal cuticle¹¹ (R.H., and A.C., unpublished) implicates these cells in melanin formation. Extensive indole quinone polymerisation would occur in the cuticle over the oenocytes if these cells did not secrete β alanine. Alternatively, a modification of the cuticular proteins secreted by the oenocytes so that they were unable to bind the indole quinones would also result in extensive melanisation, as experiments on *ebony*⁹ suggest β alanine is unable to interact with unbound indole quinones to prevent their polymerisation.

Further study is needed on the role of β alanine in cuticle maturation, for we feel that the presently accepted description of tanning and sclerotisation in many insects is incomplete.

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Spermatozoa motility in human cervical mucus

THE study of spermatozoa penetration and migration into the cervical mucus is of prime importance in the understanding of human reproductive physiology. Cervical mucus is a heterogeneous secretion, the most important constituent of which is a hydrogel made of glycoproteic mucoids¹. Changes in the arrangement of these glycoproteins seem responsible for the variation in the ability of spermatozoa to penetrate throughout the oestrous cycle. At ovulation or under oestrogenic stimulation, the macromolecules are grouped into parallel micelles which surround spaces containing a fluid of low viscosity². The penetration of spermatozoa into the mucus is entirely dependent on their own motility.

Various tests have been described to study this penetration: the post coital test provides an estimate of the spermatozoa penetration *in vivo* but only allows a very incomplete appreciation of migration intensity and of movement quality. *In vitro* tests enable a dynamic study of spermatozoa penetration into the cervical mucus to be made and a simultaneous spermiogram provides an estimate of the sperm quality.

When drops of semen and cervical mucus are placed in contact on a microscope slide, the spermatozoa form phalanges at the interface, enter the mucus and then move at random. If, however, the mucus is placed in capillary tubes, an orientated movement of the spermatozoa is observed. In these conditions the migration rate can be evaluated and the corresponding speed ranges from 1.6 to 50 $\mu\text{m s}^{-1}$ (ref. 3).

To obtain a complete, quantitative and objective evaluation of the migration of spermatozoa into cervical mucus, we have developed a method based on the Doppler effect. When an object moving at speed v receives monochromatic light having a frequency ν_0 , it scatters the light which undergoes a frequency change $\nu = \nu_0(2v/c \cos \alpha \sin(\theta/2))$ where θ is the angle between the incident beam and the direction in which the scattered light is observed, α is the angle between v and the exterior bisector of θ , and c is the speed of light. For a fixed value of θ and when v and α are single, $\nu = kv \cos \alpha$ has only one value (k being constant). When several objects move at different speeds, but in the same direction (α fixed), the frequency change gives rise to a frequency spectrum $S(\nu)$, directly representative of $N(\nu)$; N is the number of objects moving at the speed $\nu = \nu/(k \cos \alpha)$. When, moreover, α is unrestricted, the frequency change gives a more complex spectrum; in this case $N(\nu)$ is represented by the function $\nu dS(\nu)/d\nu$.

The frequency shift due to the movement of spermatozoa is very small and is detected by a heterodyne technique⁴. The incident light is a He–Ne laser beam; a phototube receives the scattered light from the moving object and a small part of the incident light simultaneously. Beats between these two lights give rise to fluctuations in the photocurrent and the analysis of them (by a Fourier analyser) gives $S(\nu)$ directly. The cervical mucus is taken with a syringe attached to a pipette made of optical quality glass which acts as a scattering cell. This pipette is 150 mm long of cross section 8×3 mm and contains along its axis a central tube 1 mm square. The fine end of the pipette is introduced into the external cervical os and the mucus is aspirated gently. The pipette is then placed for 30 min in a test tube containing 0.3 ml of the sperm to be studied.

To study migration in cervical mucus, the pipette is placed perpendicular to the incident beam which travels through it parallel to the shortest side ($e = 3$ mm), the scattered light is observed at an angle $\theta = 8^\circ$ so $\alpha = 4^\circ$ and $\cos \alpha \approx 1$. To evaluate motility of the spermatozoa in the semen itself, a flat

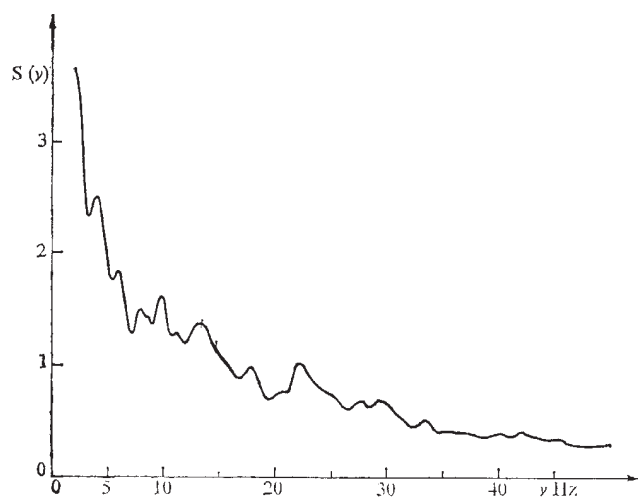


Fig. 1 Frequency spectrum $S(v)$ (arbitrary units) given by the sperm S_1 (60×10^6 spermatozoa ml^{-1} ; normal motility, 60%; reduced motility: 15%).

cell is used, 15 mm in diameter and having an optical path of 0.1 mm. The plane of the pipette is placed perpendicular to the incident beam, the power of which is less than 0.1×10^{-3} W. The temperature of the cell (pipette or plan cell) is 37°C .

Analysis of the light scattered by the spermatozoa moving in the seminal plasma gives a spectrum $S(v)$ (Fig. 1) which is wide and monotonically decreasing as v increases. The distribution $N(v_s)$ of instantaneous speed v_s in the sperm, calculated from this spectrum, is shown in Fig. 2. This wide distribution demonstrates the complexity of spermatozoa movements such as are observed under the microscope when a drop of semen is placed on a lamella.

In contrast, the spectrum representing the movement of spermatozoa in cervical mucus is a remarkably well defined line at a frequency value shifted with respect to $v = 0$ (Fig. 3A). This kind of spectrum is unambiguously significant of an

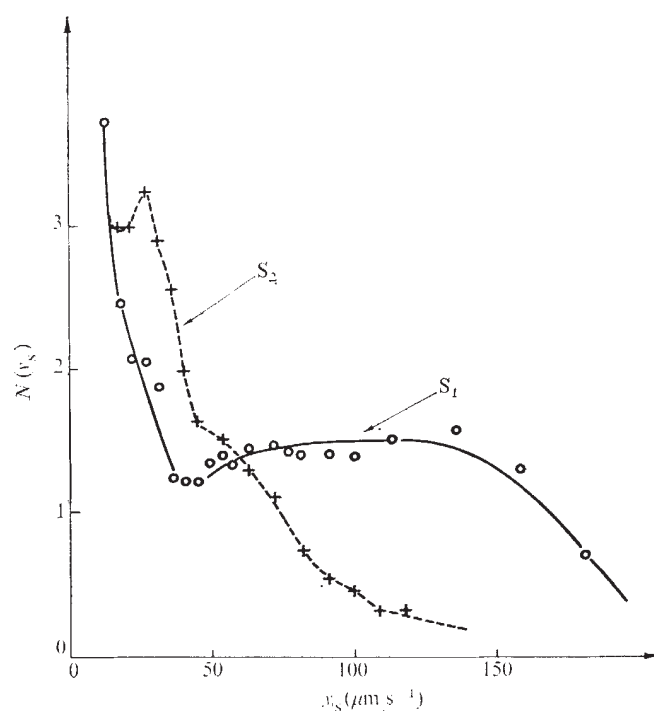


Fig. 2 Velocity distribution $N(v_s)$ (arbitrary units) against velocity v_s for sperm S_1 and S_2 (for the sperm S_2 : 94.4×10^6 spermatozoa ml^{-1} ; normal motility: 65%; reduced motility: 5%).

orientated unidirectional movement of the spermatozoa. First of all, this result strongly confirms the spermatozoa migration in cervical mucus observed under the microscope; furthermore, the spectrum gives directly the distribution of the spermatozoa speeds V_M in the mucus, the speed being related to frequency by $v = kv$. By its characteristics (high and well defined speed, $V_M = 50 \pm 10 \mu\text{m s}^{-1}$), the spectrum in Fig. 3A gives evidence of the good 'mutual' quality of the cervical mucus (orientated structure) and of sperm S_1 (high instantaneous speed of the spermatozoa).

The important part played by the sperm quality in the migration through mucus has been pointed out by a complementary experiment. The same mucus as in the previously related experiment has been penetrated by the spermatozoa of another sperm (S_2) the means speeds v_s of which, in the seminal plasma, are clearly lower than in the case of the sperm S_1 (Fig. 2). In cervical mucus, movement of the spermatozoa of sperm S_2 gives a spectrum (Fig. 3B) which is very different from that obtained with S_1 (Fig. 3A). The frequency peak is less well defined and remains in very low frequencies: its maximum corresponds to low speed V_M between 5 to $10 \mu\text{m s}^{-1}$. The relationship between the instantaneous speed v_s in the seminal plasma and

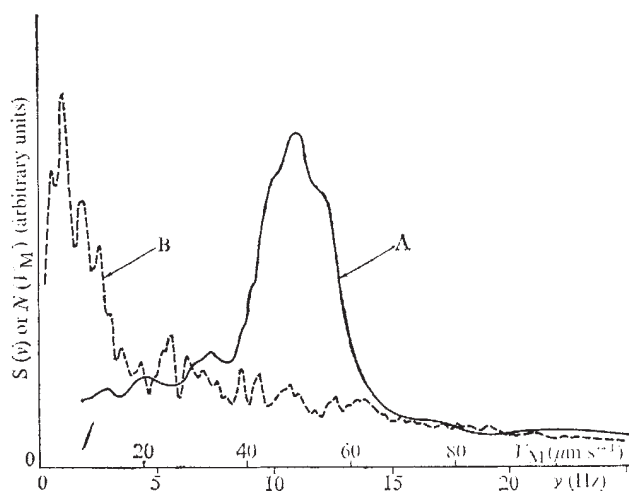


Fig. 3 Frequency spectrum (and velocity distribution) relative to the motion of the spermatozoa in cervical mucus. The measurement has been made 0.5 h after the beginning of the penetration and at 4 cm from the fine end of the pipette. A, sperm S_1 ; B, sperm S_2 .

the migration speed in the mucus is not maintained. In the case of the S_1 spermatozoa, V_s is $100 \mu\text{m s}^{-1}$, compared with V_M in cervical mucus, which is $50 \mu\text{m s}^{-1}$. For the S_2 spermatozoa, v_s is $30 \mu\text{m s}^{-1}$, when V_M in mucus is approximately estimated as $8 \mu\text{m s}^{-1}$. This indicates the functional importance of the global and objective measurement of spermatozoa velocities in cervical mucus, which points out the importance of the spermatozoa motile quality.

We believe that the scattering light technique related to the Doppler effect gives an excellent tool for the measurement of spermatozoa speeds. Objective velocimetry is of fundamental importance in the study of living motile microorganisms. If the results obtained in the particular cases of sperm S_1 and S_2 are compared by this method and by microscopic observation, the latter test indicates an apparently better quality for sperm S_2 (see Fig. legends) and this estimation, though performed by specialists, is shown to be wrong according to the objective results given by our method. In the case of migration of spermatozoa in cervical mucus, our method provides a total appreciation of the migration with a direct, simple and quick measurement of the speeds. Moreover, the results confirm that the migration is orientated at the time of

ovulation. Our study corresponds to the first observation by Doppler effect of a type of orientation of living microorganism movement, which is not due to a taxis⁵⁻⁷ but to mechanical guidance.

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Separation of parathyroid hormone and calcitonin-sensitive cells from non-responsive bone cells

BONE growth and remodelling depend on resorption and deposition at the local level. The balance between these two processes seems to be modulated by the peptide hormones PTH and calcitonin. Parathyroid hormone (PTH) has been shown to induce bone resorption both *in vivo*¹ and *in vitro*², whereas calcitonin (CT) inhibits this process³. Recent studies suggest that both agents act on cellular mechanisms through a second messenger. Thus, it has been demonstrated that exposure of mouse calvaria to PTH or CT can lead to an increase in cyclic AMP content^{4,5}. In addition, dibutyryl cyclic AMP can by itself induce bone resorption².

An understanding of the molecular mechanisms which control synthesis and degradation of bone matrix is lacking. This is a result partly of the complexity of bone tissue in which there exists within the large volume of extracellular matrix a variety of cellular types including osteocytes, osteoblasts, osteoclasts, fibrocytes and marrow elements⁶. Morphological changes suggest that bone cells can respond to both PTH⁷ and CT⁸. But, it is not known which of these cells is the primary target for the action of these hormones. Moreover, the fundamental question of whether PTH and CT act on the same or different cell types, is open. Recent efforts to isolate bone cells have resulted in mixtures of cellular types^{9,10} which have been used immediately after isolation. Attempts to maintain these cells in culture have resulted in rapid overgrowth by fibroblasts⁹.

A clear elucidation of the molecular events triggered by PTH and CT should be aided by the isolation and identification of the various cellular types. Here we report the isolation of a selected population of bone cells from mouse calvaria which exhibit and retain in culture for several days distinct morphological and biochemical characteristics.

Freshly excised calvaria (usually 25 in number and weighing about 100 mg) were minced and extracted by stirring for 20 min in an isotonic, calcium-free salt solution containing 0.1% collagenase, 0.05% trypsin and 0.005 M EDTA (Table 1). The cells released into the extract were collected by centrifugation at 10,000 r.p.m. for 5 min (population 1). This extraction was repeated a second and a third time to yield cell populations 2 and 3. The yields of cells were 1×10^6 , 2×10^6 and 2×10^6 in populations 1, 2 and 3 respectively. DNA measurements

established that 20% of the total calvarial DNA had been extracted. Samples of calvaria were examined histologically with haematoxylin and eosin and Von Kossa stains after each step of the extraction procedure. There was a high degree of variability in appearance from sample to sample and within the same sample of tissue. The general impression was that the extraction progressively removed cells from the periosteal surface of the calvaria inward such that there was an increasing number of empty lacunae. After the third extraction osteocytes buried in the calcified matrix still remained, as did most of the cartilage and chondrocytes. These remaining cells comprised the bulk of those present in the control samples in agreement with the analyses for DNA.

Cells of each population were placed in monolayer culture at a density of 2×10^6 cells per culture flask. In addition, the calvarial tissue remaining after the third extraction was also placed in culture. Populations 1, 2 and 3 all exhibited a similar morphology immediately upon isolation. This similarity persisted for up to 48 h in culture and appeared as a mixture of small rounded and stellate cells. After 5-6 d, differences in morphology and growth pattern became evident in the three populations. Population 1 rapidly divided and by 7-10 d spindle shaped cells resembling fibroblasts predominated in the culture (Fig. 1a). The morphology of population 3, in contrast, was transformed into relatively large cells with a highly convoluted dendritic border (Fig. 1b). Cellular division was slow and this morphology persisted for at least 2 weeks. Population 2 at first exhibited morphological characteristics of both populations 1 and 3, but by the 14th day the spindle shaped cells had overgrown the culture. In the case of the cultured calvarial remnants, cells migrated out from the tissue (population 4). Morphologically, these cells resembled closely those of population 3 in their stellate appearance but were somewhat larger. These cells also maintained their morphology on extended culture.

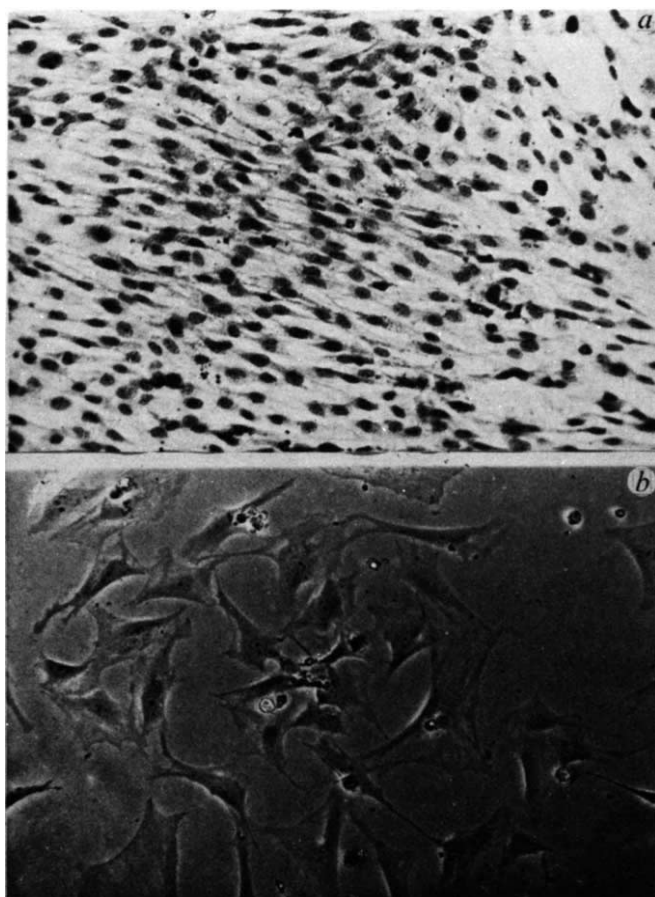


Fig. 1 Photographs of bone cells population 1 (a) (fixed and stained in haematoxylin and eosin) and population 3 (b) 7 days after extraction from mouse calvaria ($\times 200$).

Table 1 Response of bone cell populations to PTH and CT

Sample	Control	Cyclic AMP (pmol) per 100 µg DNA		PTH 0.44 U ml ⁻¹ SCT 0.25 U ml ⁻¹
		PTH 0.44 U ml ⁻¹	SCT 0.25 U ml ⁻¹	
Unextracted calvaria	25 ± 1	227 ± 4*	50 ± 5*	248 ± 4*
Extracted calvarial remains	28 ± 4	48 ± 6*	32 ± 4	48 ± 3*
Population 1	20 ± 3	21 ± 3	15 ± 2	19 ± 3
Population 2	21 ± 2	125 ± 5*	38 ± 4*	141 ± 6
Population 3	24 ± 4	236 ± 7*	65 ± 7*	260 ± 7
Population 4	27 ± 3	56 ± 3*	32 ± 5	57 ± 6*

Calvaria from 2-3-d-old mice were minced and suspended with constant stirring in 5 ml of a sterile salt solution consisting of 136.0 mM NaCl, 2.6 mM KCl, and 0.36 mM NaH₂PO₄, containing 0.1% collagenase (180 U mg⁻¹, Worthington Crude), 0.05% trypsin (BBL lyophilised powder 2.5%) and 5 mM EDTA, pH 7.0. The cells released into solution after 20 min were collected by centrifugation at 10,000 r.p.m. for 5 min at 20°C (population 1). Fresh enzyme solution was added to the calvarial remains and the procedure was repeated twice to give to cell populations 2 and 3. These were placed in monolayer culture in Minimal Eagles Medium nutrient mixture (Gibco) supplemented with 10% foetal calf serum (Gibco), 100 U ml⁻¹ penicillin, 100 mg ml⁻¹ streptomycin and 2.2 mg ml⁻¹ Na bicarbonate. The calvarial remains were either assayed immediately for hormonal response or were placed in culture to yield population 4. After 7 d in culture, 5 × 10⁵ cells from each cell population were inoculated into culture dishes. The standard growth medium was decanted 6 h later and fresh medium containing 5 mM theophylline and PTH (0.44 U ml⁻¹) or SCT (0.25 U ml⁻¹) was added to the monolayers. The reaction was stopped 5 min later with two rapid rinses in ice cold Tyrode's salt solution. 0.8 ml of ice cold ethanol-0.2 N HCl was added, and the cells were collected by scraping the culture dishes. After 24 h at -10°C the extracts were centrifuged. The supernatants were removed, dried *in vacuo*, and cyclic AMP measured by the method of Gilman¹¹. Protein kinase and protein kinase inhibitor were obtained from Sigma. All determinations were performed in duplicate at two different concentrations of cyclic AMP. Known amounts of cyclic AMP were added to aliquots of each extract as internal standards, and recovery was more than 90%. The pellets were heated in 10% perchloric acid and DNA was measured by Burton's modification of the diphenyl reaction¹². Each number in the table represents the average ± s.e.m. of 6-8 separate studies.

*Differs significantly from control $P < 0.01$.

The four cell populations and fresh unextracted and extracted calvaria were tested for their ability to produce cyclic AMP in response to the bone-active hormones PTH and CT (Table 1). As previously established^{4,5}, both hormones rapidly elicited severalfold increases in cyclic AMP content of intact calvaria. The response to PTH was about fourfold greater than to CT at maximally effective concentrations. The effects of the two hormones were additive. After the removal of populations 1-3, however, the calvaria responded only minimally to PTH and not at all to CT suggesting that essentially all of the hormone-sensitive cell populations had been removed by the extraction procedure. Among the cultured isolated bone cells, population 3 exhibited the greatest responsiveness to both PTH and CT. Populations 2 and 4 were substantially less responsive, and population 1 was not responsive. The ratio of the increases in cyclic AMP produced by each hormone was about the same in the responding cell populations as it was in the fresh calvaria. Moreover, the effects of the two hormones were additive.

The action of a few other hormones on the separated cell populations was examined (Table 2). ACTH and MSH had no effect on cyclic AMP levels on the three populations, whereas PTH, CT and adrenaline (which has been reported to affect the level of cyclic AMP in bones¹⁰) all increased the cellular content of this nucleotide in population 3 and to a smaller extent in population 2. As before, population 1 was unresponsive.

On extended culture, the maximum responsiveness of population 3 to both PTH and CT individually and in combination

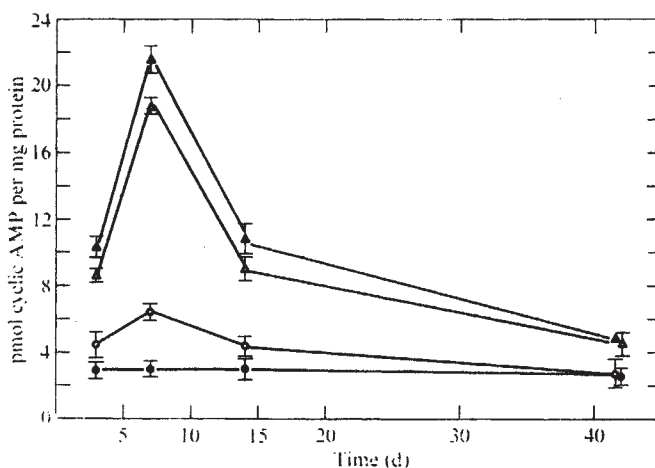


Fig. 2 Loss of response to PTH and CT upon prolonged culture of population 3. At various times after establishment in culture, 2 × 10⁵ bone cells from population 3 were grown under standard conditions with no additions, (●); PTH 0.44 U ml⁻¹, (Δ); SCT 0.5 U ml⁻¹, (○) or PTH (0.44 U ml⁻¹) and SCT (0.5 U ml⁻¹), (Δ·) in media supplemented with 5 mM theophylline. After 3 min the reaction was stopped by rapid rinsing of the cells with ice cold Tyrode's salt solution. The cells were then processed for cyclic AMP measurements as described in Table 1. Protein was measured by the method of Lowry *et al.*¹³. The bars for each data point represent ± s.e.m.

Table 2 Specificity of hormonal response

Agent	Cyclic AMP (pmol) per 100 µg DNA			
	1	2	3	4
None	48 ± 7	28 ± 4	22 ± 2	22 ± 5
PTH (0.44 U ml ⁻¹)	40 ± 10	49 ± 10*	161 ± 20*	41 ± 10*
SCT (0.5 U ml ⁻¹)	34 ± 2	37 ± 3†	65 ± 7*	28 ± 3
L-adrenaline (5 × 10 ⁻⁶ M)	39 ± 1	31 ± 2	71 ± 13*	42 ± 5*
ACTH (0.35 U ml ⁻¹)	40 ± 2	20 ± 5	25 ± 3	18 ± 2
MSH (10 ⁻⁸ M)	35 ± 3	25 ± 4	30 ± 2	21 ± 3

Specificity of hormonal response. Seven days after establishment in culture, bone cell populations 1, 2, 3 and 4 were subcultured in 5 mM theophylline and PTH (0.44 U ml⁻¹), ACTH (0.35 U ml⁻¹), MSH (10⁻⁸ M), SCT (0.5 U ml⁻¹) or L-adrenaline (8 × 10⁻⁶ M) was added for 10 min. The control medium contained only theophylline. The reaction was stopped by the addition of ice cold Tyrode's salt solution. Cells were rinsed and processed for cyclic AMP measurement as described in Table 1. MSH was a gift from Dr J. Pawalek, and ACTH and L-adrenaline were purchased from Sigma.

* Differs significantly from control $P < 0.01$

† Differs significantly from control $P < 0.05$.

reached a peak at 7 d and then declined (Fig. 2). After 40 d the response to PTH was slight and that to CT was not detectable. Peak responsiveness occurred at the time when the distinctive morphology illustrated here (Fig. 1b) was well established.

These results indicate that a selected population of bone cells may be obtained by sequential extraction which seem to be specific targets for PTH and CT. To our knowledge this is the first time that such a separation has been demonstrated.

The present data which show an additive effect of PTH and CT on cyclic AMP levels suggest that non-competing receptor sites for both hormones are present in population 3. The co-extraction of PTH and CT-responsive cells (Table 1), the simultaneous peaking of the responses at one week in culture (Fig. 2), and the simultaneous loss of this responsiveness on extended culture (Fig. 2) suggest that the receptors to PTH and CT reside either in the same cell, or in different cells which exhibit a similarity in their extractability from bone and viability in culture. Efforts are under way to further fractionate the cells in population 3 (on the assumption that this is a heterogeneous population), and to investigate some of the molecular events initiated by these hormones.

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Presence of a gonadotrophin on the surface of preimplanted mouse embryos

IN humans, a gonadotrophin (hCG) is localised on the cell membrane of the syncytiotrophoblast facing the maternal bloodstream¹. The syncytiotrophoblast is derived from the trophoblast cells of the mammalian blastocyst and is thought to participate in isolating the foetus from attack by the maternal immunological system². Martin, *et al.*¹ suggest that the hCG on human syncytial plasma membranes may, in part, be responsible for the lack of maternal immunological rejection of the foetus; in addition to its role in steroidogenesis, therefore, hCG may also have an immunological function during pregnancy. Because hCG is generally used to induce super-ovulation in mice and must, therefore, cross-react with mouse gonadotrophin, and since the mouse is extensively used in studies on

mammalian development, I investigated the possible presence of gonadotrophin(s) in mouse embryos before implantation and formation of the syncytiotrophoblast. Using an antiserum to hCG and indirect immunofluorescence (IIF) I have detected a luteinising hormone (LH)- or hCG-like substance ('embryonic gonadotrophin', EG) on the cell surfaces, of live mouse pre-implanted embryos.

Random-bred SWR female mice 6-8 weeks old were super-ovulated and mated to provide unfertilised eggs and various *in vivo* preimplantation stages for the IIF assays³. Super-ovulated BALB/c females mated with C57BL males, and naturally-ovulated ICR female mice mated with C3H males, provided additional *in vivo* morulae and blastocysts. Two-cell embryos from super-ovulated SWR mice were collected and cultured to blastocysts, using standard techniques^{4,5}, to provide *in vitro* preimplantation stages. Blastocysts which had been collected from SWR mice and allowed to attach to, and grow on, glass slides were also examined. The IIF assay consisted of a 30-min incubation in rabbit anti-hCG (Miles Laboratories) diluted 1:1 with phosphate buffered saline (PBS), two 5-min rinses in culture medium, a 10-min incubation in goat anti-rabbit IgG conjugated with fluorescein (Antibodies, Inc.) diluted 1:4 with PBS, and two 5-min rinses in culture media to remove unbound conjugate. Slides of fixed mouse placenta (16-18 d), human second-trimester placenta, and mouse ovary were assayed for IIF-detectable gonadotrophin using anti-hCG and anti-rabbit IgG fluorescein conjugate as previously described⁶. IIF controls were incubated in either normal rabbit serum followed by fluorescein, or in the fluorescein conjugate only. Most of the stages were assayed in triplicate. Embryos were placed in a small amount of culture medium on glass slides, and the slides of tissue sections were viewed with a Zeiss fluorescence microscope and photographed using Agfa GAF 500 colour slide film (exposures of 1 min).

Before the eight-cell stage, little gonadotrophin was detected on the cell surface on any of the stages (Fig. 1). Then, only a few isolated blastomeres of an occasional embryo would show light fluorescent outlines. Cell surfaces of morulae, however, were strongly positive for gonadotrophin and remained positive as blastocoel formation ensued. With blastocyst expansion, however, fluorescence diminished to pre-morulae levels and was not detectable on blastocysts which had attached to, and grown on, glass. Results for embryos cultured from the two-cell stage were similar except that the marked increase in positive fluorescence observed with the morulae was depressed slightly and persisted throughout blastocyst hatching. No consistent differences in the pattern of positive fluorescence was observed between embryos taken from naturally or super-ovulated mice,

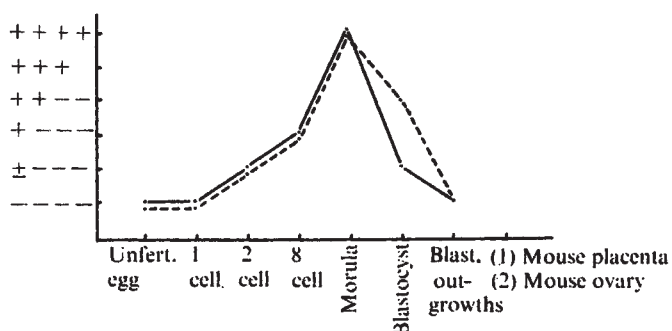


Fig. 1 Cell-surface gonadotrophin detected by IIF on mouse preimplantation embryos. The impressions of three observers who scored the embryos for positive fluorescence were averaged to produce the differences in fluorescence assigned to the pre-implantation stages. The symbols (---) and (++++), represent the total lack, and the greatest amount, respectively, of IIF-detected cell-surface gonadotrophin. Gradual replacement of plus signs with minus signs indicate diminishing levels of fluorescence. *In vivo* embryos, ———; cultured embryos, -----.

or from mice of different strains. No anti-hCG binding was observed on mouse 16–18 d gestation placenta, or mouse ovary. Positive fluorescence was observed on sections of human second-trimester placenta, and was confined to the syncytiotrophoblast of the chorionic villi.

Existing opinion holds that production of EG (gonadotrophin secreted by the placenta or its derivatives) is confined to the syncytiotrophoblast. The rabbit blastocyst has, however, been shown to contain a gonadotrophin which competes with hCG in binding assays⁷, and to secrete an LH-like substance by radioimmunoassay⁸. These observations, together with the high maternal plasma levels of hCG observed at about the time of implantation⁹, indicate that EG may be produced before implantation and formation of a syncytium. The observation that anti-hCG binds to morulae cultured from two-cell embryos suggests that, in addition to having a trophic hormone(s) on their cell surfaces (irrespective of the origin of the hormone), mouse preimplantation embryos may synthesise at least some of the gonadotrophin found on their surfaces.

Besides initiating steroidogenesis, cell-bound gonadotrophins may play a role in mediating protection of the conceptus against maternal rejection during implantation. Such a role has been suggested for hCG, which reversibly inhibits PHA-stimulation of human lymphocytes *in vitro*¹⁰ and is found in high concentrations in plasma throughout pregnancy. It is not known whether EG has a similar role in rodents nor whether chorionic gonadotrophin is present in rodents, including the mouse (A. Contopoulos, personal communication). No anti-hCG binding was detected on blastocyst outgrowths, nor on mouse placenta, whereas human placenta similarly fixed and assayed was positive for anti-hCG binding. Perhaps a gonadotrophin is present in mouse placenta, but in a form not recognised by anti-hCG.

It is now widely thought that polypeptide hormones achieve their effects by first interacting with the cell membrane or with membrane-bound enzymes¹¹. hCG binds to the cell membranes of luteinised rat ovaries and presumably such binding precedes the onset of ovarian steroidogenesis¹². Initiation of steroidogenesis found in the preimplantation embryos of the rat¹³ and rabbit¹⁴ may also require the presence of cell surface-bound gonadotrophin. In addition, it has been suggested that steroidogenesis in the preimplanted rat embryo may be necessary for morulae to blastocyst transformation¹³. Similar processes may also occur in the mouse.

In conclusion, mouse preimplantation embryos display a gonadotrophin(s) on their cell surfaces, and IIF-detectable levels of this gonadotrophin(s) is maximal on morulae. In addition, I suggest that the preimplanted mouse embryo may be capable of synthesising at least some of the gonadotrophin found on their surfaces.

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1,25-dihydroxycholecalciferol-like activity of *Solanum malacoxylon* extract on calcium transport

CATTLE grazing the South American egg plant, *Solanum malacoxylon*, develop hypercalcaemia and hyperphosphataemia resulting in severe calcosinosis¹. The active principle in the plant stimulates intestinal calcium transport^{2–4} and bone mineral resorption^{2–4} in laboratory animals. It resembles vitamin D except that it is water soluble^{1,5} and exerts its initial action and declines in activity more rapidly². Recent work⁶ suggests that the factor closely resembles 1,25-dihydroxycholecalciferol (1,25-(OH)₂-D₃), a metabolite of vitamin D₃ formed in the kidney⁷, in that it overcomes the inhibition by dietary strontium⁸ of vitamin D₃-induction of the intestinal calcium-binding protein (CaBP) and, concomitantly, calcium absorption⁹.

Here we demonstrate that the *S. malacoxylon* factor stimulates the calcium absorptive mechanism of the organ-cultured chick duodenum, indicating that the activity of the factor does not depend on preliminary metabolism by some other tissue, that, indeed, it resembles 1,25-(OH)₂-D₃ in terms of its biopotency, and that its mechanism of action, like that of both vitamin D₃ itself and 1,25-(OH)₂-D₃, is dependent on intestinal protein synthesis.

Technical details of the organ-culture system, assay of CaBP and measurement of calcium uptake and transport in the cultured duodenum have been published elsewhere^{10–12}.

After preliminary experiments proved that *S. malacoxylon* extract (Fig. 1 legend) was effective in eliciting vitamin D-like responses in organ-cultured duodena, a time study was conducted (Fig. 1). Within 12 h of culture, the extract induced CaBP synthesis and stimulated ⁴⁵Ca uptake. There was excellent correlation between CaBP concentration in the tissue and the ability of the tissue to accumulate radiocalcium. This experiment confirmed the vitamin D-like activity of the *Solanum* factor as only vitamin D₃, and related sterols^{10–13}, are effective in inducing CaBP in this system. The lag time observed was more reminiscent of vitamin D₃ itself (which is effective without transformation in this system¹¹) than 1,25-(OH)₂-D₃ (refs 10 and 13). There is, however, ample evidence (for example, its water solubility^{1,5}) that the active principle in *S. malacoxylon* is not a simple sterol (Peterlik and R.H.W., unpublished). The possibility exists, therefore, that some preliminary transformation of the principle must take place before its characteristic activity ensues but our results indicate that such change can occur in the intestine itself.

Other evidence supports the contention that the *Solanum* factor is more like 1,25-(OH)₂-D₃ than vitamin D₃. The dose-response relationship for *Solanum* extract was compared with that of authentic 1,25-(OH)₂-D₃, by far, the most potent sterol for CaBP induction in this system^{10,11}. In terms of CaBP-inducing potency, 0.01 and 0.1% concentrations of *Solanum* extract were equipotent to 65 and 650 pM 1,25-(OH)₂-D₃, respectively (Table 1). This means that there were 650 pmol of 1,25-(OH)₂-D₃-like activity in each ml of *Solanum* extract, or 365 pmol of 1,25-(OH)₂-D₃-like activity per g of powdered leaf.

Another experiment confirmed the 1,25-(OH)₂-D₃-like

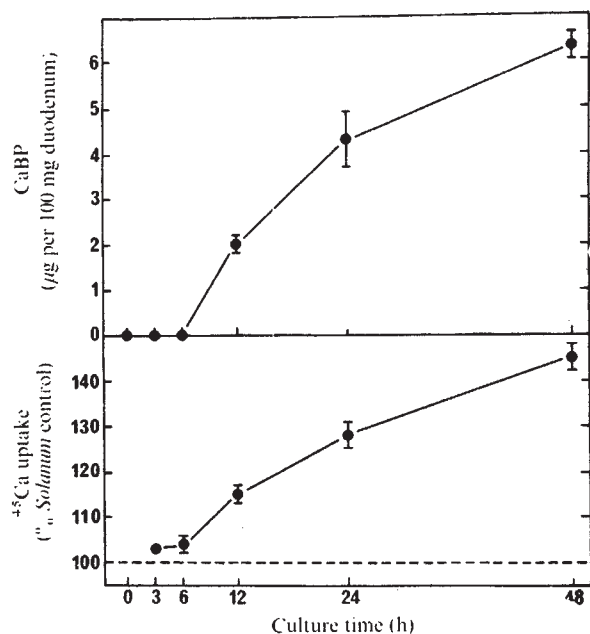


Fig. 1 Time study of the effect of *S. malacoxylon* extract (0.1% of the culture medium) on CaBP synthesis and stimulation of ^{45}Ca uptake. Values are the mean $\pm$ s.e. The *S. malacoxylon* extract used in these experiments was prepared by shaking the dried, ground leaf three times with three volumes of methanol: chloroform (2:1) for 2 h at room temperature discarding the solvent each time. After residual solvent removal by aeration, the residue was shaken with nine volumes of water for 16 h. The aqueous extract was filtered through cheese-cloth and centrifuged (24,000g for 20 min). The supernatant was lyophilised, shaken for 16 h in 75% ethanol in water and centrifuged. The ethanolic extract was concentrated sixfold and stored at -20°C . This procedure effected a 42-fold concentration of the active principle over the powdered leaf. Activity of the various fractions was assayed by determining CaBP production when administered to rachitic chicks 4 weeks old¹⁸. Aliquots of this final concentrated extract were added to the culture medium as indicated. Duodena were cultured slit-open on grids^{10,11,13} mucosal-side-up for the times indicated, in the presence of either the extract or extracting solvent alone (75% EtOH-control group) in the culture medium. CaBP assay and ^{45}Ca uptake were performed as previously described^{10,13}. There was no CaBP present in duodena cultured in the presence of extracting solvent alone.

activity of the *Solanum* factor in what has proven to be a new test system, that is, the embryonic chick duodenum *in ovo*. Some time ago, it was found that embryonic chick duodenum neither contains CaBP until the day of hatching (day 21) nor synthesises CaBP in response to the injection of vitamin D_3 directly into the egg¹⁴. Recent work has confirmed this original observation while demonstrating that metabolism of vitamin D_3 to its more active metabolites does occur in the embryo¹⁵. We here report that injection of $1,25\text{-(OH)}_2\text{-D}_3$ (300 pmol per egg) directly into the egg (at 18 d incubation) did induce precocious CaBP synthesis when measured 36 h after injection (8.3 ± 0.9 μg CaBP per 100 mg duodenum). Interestingly, when *Solanum* extract was injected into each egg (an amount estimated to contain 325 pmol $1,25\text{-(OH)}_2\text{-D}_3$ -like activity; see Table 1), CaBP was also induced and to an almost theoretically predictable extent (8.9 ± 1.7 μg CaBP per 100 mg duodenum). By contrast, 240,000 pmol vitamin D_3 (800 times as much $1,25\text{-(OH)}_2\text{-D}_3$ on a molar basis) was completely without effect and 26,000 pmol 25-OH-D_3 was only marginally effective (3.2 ± 0.6 μg CaBP per 100 mg duodenum). The embryonic chick duodenum is refractory to endogenous levels of vitamin D_3 and its metabolites, although possessing the inherent ability to respond to sufficient levels of these sterols both in organ culture¹⁰⁻¹³ and, to the active metabolites, *in ovo*. This strongly suggests that stimulatory levels of the appropriate sterol are not reached in the duodenum *in ovo*. The reason for this is completely unknown.

The action of either vitamin D_3 (refs 12 and 13) or $1,25\text{-(OH)}_2\text{-D}_3$ (ref. 16) in the organ cultured duodenum has been shown to be an actinomycin D-sensitive process, that is, sterol action involves DNA transcription and subsequent protein synthesis. The *Solanum* factor seems to act by a similar mechanism as actinomycin D completely abolished the response to *Solanum* extract (Table 2). It is also interesting to note that not only ^{45}Ca uptake by the duodenum, but also mucosal-to-serosal transport were stimulated by *Solanum* and that both ^{45}Ca movements were inhibited by actinomycin D.

The foregoing experiments confirm and amplify previous work⁶ indicating the $1,25\text{-(OH)}_2\text{-D}_3$ -like activity of the *Solanum* factor. There is no doubt that this factor functions by an actinomycin D-sensitive mechanism, similar to that of the D-vitamins, in inducing CaBP and stimulating calcium transport. There also can be little doubt that, with respect to its biopotency in culture and *in ovo*, the factor in *S. malacoxylon* extract resembles $1,25\text{-(OH)}_2\text{-D}_3$. The recent report that aqueous extracts of *Solanum* failed to mobilise bone calcium in vitamin D-deficient rats fed a low calcium diet, while $1,25\text{-(OH)}_2\text{-D}_3$ was effective¹⁷, is hard to reconcile with the present findings, those with strontium-fed chicks⁶, and those of earlier workers who did, in fact, show an action on bone mineral resorption^{2,4}.

Table 1 Relative potency of *S. malacoxylon* extract and $1,25\text{-(OH)}_2\text{-D}_3$ in the induction of CaBP

Concentration in medium		CaBP per 100 mg duodenum (μg)
<i>S. malacoxylon</i> Extract (%)	$1,25\text{-(OH)}_2\text{-D}_3$ (pM)	
0 (EtOH only)	0	0
0.01	—	3.5 ± 0.6
0.1	—	7.8 ± 0.7
—	65	3.4 ± 0.5
—	650	7.4 ± 0.5

Values are mean $\pm$ s.e. Duodena were cultured slit-open on grids^{10,11,13} mucosal-side-up for 48 h in the presence of the extract (prepared as given in the legend to Fig. 1) or the authentic sterol in the culture medium. Additions were made in EtOH such that the final EtOH concentration was 0.1%. CaBP assay was performed as previously described¹⁰⁻¹³.

Finally, the active principle from *Solanum malacoxylon*, or, perhaps, from some abundant native plant, holds promise as a source of $1,25\text{-(OH)}_2\text{-D}_3$ -like activity. This may prove important given the difficulty and expense of producing synthetic $1,25\text{-(OH)}_2\text{-D}_3$ and the widespread desirability of utilising $1,25\text{-(OH)}_2\text{-D}_3$ for studies of animal and human disorders of calcium metabolism which may be refractory to vitamin D_3 itself. Isolation and purification of the active principle is of great importance and is proceeding in several laboratories. In our own laboratory, the organ culture system—and the *in ovo*

Table 2 Inhibition of the action of *S. malacoxylon* extract on CaBP synthesis and radiocalcium uptake and transport in organ-cultured duodenum by actinomycin D

Concentration in medium		^{45}Ca movement (% of zero extract control)	
<i>S. malacoxylon</i> extract (0.1%)	Actinomycin D (1.6 μM)	CaBP per 100 mg duodenum (μg)	Tissue uptake Mucosal $\rightarrow$ serosal transport
—	—	0	100
+	—	2.9 ± 0.3	$145 \pm 5^*$
+	+	0	$176 \pm 12^*$
+	+	0	100
+	+	0	97 ± 4
+	+	0	107 ± 12

Values are the mean $\pm$ s.e. Duodena were cultured everted on grids^{10,13} for 48 h in the presence of *Solanum* extract (Fig. 1 legend) and actinomycin D in the culture medium. CaBP assay and measurement of ^{45}Ca movement were performed as previously described^{10,13}.

*Significantly greater than zero extract control at 1% level; Students' *t* test.

preparation—has proved to be of significant value in quantifying the factor during the various stages of its isolation.

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Malaria and the permeability of the host erythrocyte

WHEN an animal is infected with malaria some of the properties of the membrane of its erythrocytes are changed¹. The fragility is increased^{2–5} and the transport of sodium, potassium and amino acids across the membrane is altered^{6–10}. In most cases these alterations have been shown to be present in all erythrocytes from an infected animal and not just those containing parasites, which suggests a general pathological effect rather than a direct action of the parasite on its host cell.

We have found that erythrocytes from infected animals also show an increased permeability to L-glucose, a substance which does not readily penetrate either the membrane of erythrocytes from normal animals or that of immature red cells. This increased permeability could be related to the other membrane changes described above, and if so should be shown by both parasitised and non-parasitised erythrocytes from an infected animal. The complete separation of these two types of cell would be difficult, but an approximate separation can be made by centrifugation^{5,6,8}, since those cells which contain parasites sediment more slowly than those which do not. We have therefore used this technique to determine whether all cells or only parasitised cells show an increased permeability to L-glucose.

Blood from 10–15 mice infected with *Plasmodium berghei* (a malarial parasite of rodents) was pooled, the white cells removed and the erythrocytes collected by centrifugation after washing

three times with buffer medium (pH 7.35). Approximately the upper and lower quarters of the packed cells were removed, each resuspended in buffer medium containing L-1-¹⁴C-glucose, and centrifuged after 3 min. Radioactivity in cells and buffer medium was then measured in a liquid scintillation counter. Details of the materials and general methods used will be published elsewhere (K. D. Neame and C. A. Homewood, unpublished). The possibility that the pooled blood contained erythrocytes from uninfected or poorly infected animals was avoided by examining blood from each animal, and rejecting any animals in which the percentage of erythrocytes parasitised was particularly low for the group.

The uptake of the labelled L-glucose by the erythrocytes is shown in Fig. 1. Uptake has been expressed in terms of the distribution space in the centrifuged packed cells, which included residual buffer medium. The inulin space of packed erythrocytes is therefore also given, and the L-glucose space of erythrocytes from normal animals is included in the figure for comparison. It can be seen that the parasite-rich fraction of cells (top layer of packed cells) showed a consistently higher L-glucose space than did the parasite-poor fraction (bottom layer), and that the size of the L-glucose space was proportional to the percentage of erythrocytes which were parasitised.

These findings strongly suggest that only those cells which contain parasites become readily permeable to L-glucose, and that the change in permeability thus appears to be a direct effect of the parasite on the cell in which it is contained, and is not merely a generalised effect on all erythrocytes. This is supported by the further finding that incubation (at 37° C for 1 h) of normal erythrocytes with plasma from infected mice had no effect on their subsequent permeability to L-glucose.

This increase in the permeability of the parasitised erythrocyte may be a striking example of the means by which a parasite, in addition to adapting itself to the host, might also adapt the host cell to its own requirements by allowing the entry into the erythrocyte of certain nutrients normally excluded from the cell.

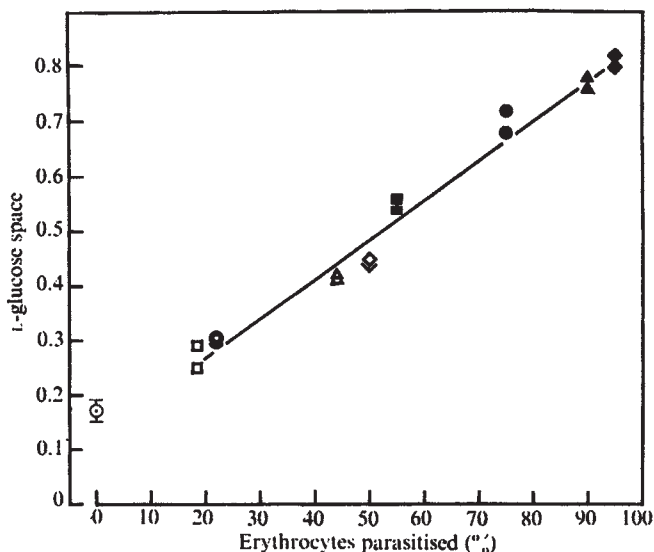


Fig. 1 Relationship between uptake of L-glucose by erythrocytes and percentage of erythrocytes parasitised. Erythrocytes from mice infected with *P. berghei* were fractionated as described in the text. Each group of four symbols of similar shape gives values from a single pooled sample of blood; the upper pair (closed symbols) represent duplicate samples from the top layer of centrifuged cells, and the lower pair (open symbols) represent duplicate samples from the bottom layer. Inulin space of packed cells: normal mice, 0.14 ± 0.02 s.d., $n = 6$; mice with about half the erythrocytes parasitised, 0.19 ± 0.02 s.d., $n = 8$. $\odot$, L-glucose space of erythrocytes from uninfected mice (mean $\pm$ s.d., $n = 6$).

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Tyrosine-dependent increase of tyrosine hydroxylase in neuroblastoma cells

TYROSINE hydroxylase (tyrosine-3-monooxygenase), presumably the rate-limiting enzyme in the biosynthesis of the adrenergic transmitters dopamine and noradrenaline^{1,2}, catalyses the hydroxylation of both phenylalanine and tyrosine^{3,4}. This property has been exploited for the selection of adrenergic-like mouse neuroblastoma cells⁵, which have high tyrosine hydroxylase activity and can grow in the absence of tyrosine because they can convert sufficient phenylalanine to tyrosine for cell growth. N1E-115 is such a neuroblastoma clone^{5,6} and we have now found that, when the amount of tyrosine in the medium is increased, there is an increase in the amount of tyrosine hydroxylase in N1E-115 cells. Modification of the amount of this rate-limiting enzyme by its substrate concentration may play a fundamental role in the regulation of the biosynthesis of catecholamines.

N1E-115 cells (provided by Dr Marshall Nirenberg) were grown as monolayer cultures in polystyrene Petri dishes, 150 mm in diameter (Falcon), at 37° C in a humidified atmosphere of 10% CO₂ and 90% air. Cells were grown as before⁷ in the Dulbecco-Vogt modification of Eagle's medium (DNEM, Gibco, catalogue No. H-21) containing 4.5 g of dextrose per litre, 0.42 mM tyrosine and phenylalanine, no Na pyruvate and 5% foetal calf serum. Cells were maintained in stationary phase for approximately 1 week and fed a mixture of 3 parts F-14 medium⁸ and 1 part DNEM which contained a final concentration of 0.16 mM tyrosine and 0.21 mM phenylalanine. During experimental procedures, cells were maintained in F-14 medium containing 0.12 mM phenylalanine, various concentrations of tyrosine and 2% dialysed foetal calf serum. After experimental incubations monolayers were washed three times with an isotonic salt solution and cells were collected by scraping and washing with approximately 1 ml of 0.05 M potassium phosphate buffer (pH 6.8) containing 8% sucrose. The recovered suspension was sonicated and centrifuged at 30,000g for 30 min. The clear extract that we used contained more than 90% of the total tyrosine hydroxylase activity. Tyrosine hydroxylase activity was measured by the tritium release method⁹. The standard assay was carried out in 0.50 ml that contained: 50 µmol potassium phosphate (final pH 6.1); 25 nmol L-tyrosine containing 800,000 c.p.m. of 3,5-³H-L-tyrosine (original specific

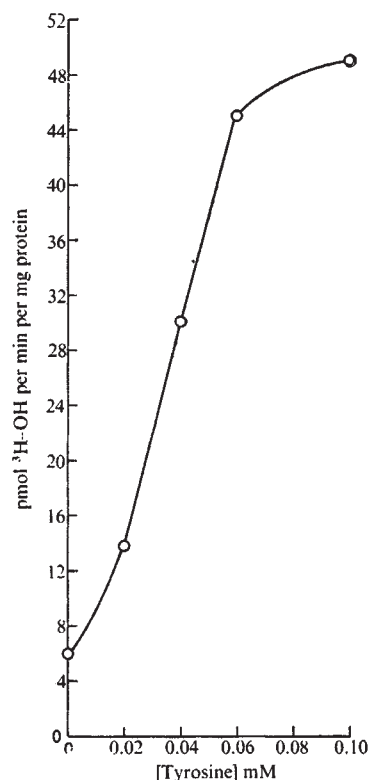


Fig. 1 Effect of increasing levels of tyrosine in the culture media on tyrosine hydroxylase activity in N1E-115 neuroblastoma cell extracts.

activity 1.0 Ci mmol⁻¹) (Amersham-Searle) purified before use¹⁰, 20 µmol β-mercaptoethanol, 2,000 U catalase (Boehringer-Mannheim), 0.50 µmol 6-methyltetrahydropterin (Calbiochem) and the tyrosine hydroxylase-containing extract to be assayed (0.3–0.7 mg protein per assay). Incubations were carried out at 37° C for 10 min and stopped with 0.5 ml of 10% trichloroacetic acid. Protein was determined by the method of Lowry *et al.* with bovine serum albumin as a standard¹¹. The tyrosine hydroxylase activity from this cell line has been reported to be higher than the activity described here¹². Several variables may account for this difference, for example: (1) previous use of 10% undialysed foetal calf serum, (2) growth of cells to a greater degree of differentiation before and (3) use of 500 µM tyrosine in the earlier assay system compared with the use of 50 µM tyrosine in the present assay system. Specific tyrosine hydroxylase anti-serum was produced in sheep as before¹³. A crude γ-globulin fraction was obtained by fractionating control and immune sera by the addition of saturated ammonium sulphate at pH 6.8 until 25% saturation was achieved. The pellet was collected by centrifugation and resuspended in the original volume of serum with 0.02 M potassium phosphate and 0.3 M glycine buffer, pH 6.8. The 0–25% ammonium sulphate fractionation was repeated and the second pellet was suspended in the same volume of phosphate-glycine buffer.

When N1E-115 cells were incubated for 24 h in media containing tyrosine, the activity of tyrosine hydroxylase increased eightfold as tyrosine concentration increased from 0 to 0.10 mM (Fig. 1). In other experiments (not shown) the increase in tyrosine hydroxylase activity levelled off at concentrations of 0.10–0.30 mM tyrosine. Results were similar with another adrenergic-like neuroblastoma cell line N-TD6⁸. During the 24 h exposures to various concentrations of tyrosine, total cellular protein per dish did not vary more than 15%. Total and specific tyrosine hydroxylase activity in cells maintained without tyrosine for 24 h and then exposed to 0.10 mM tyrosine for 0, 1, 4, 9, 13 and 24 h, increased in a time-dependent manner (Fig. 2). After 24 h this enzyme activity had increased threefold. It is not apparent why a larger increase was not observed.

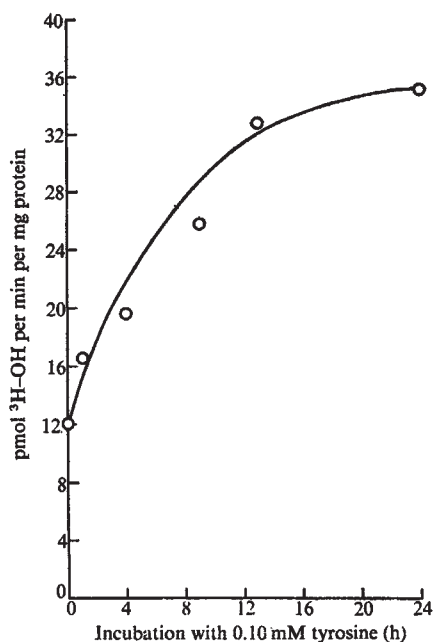


Fig. 2 Increase in tyrosine hydroxylase activity in NIE-115 neuroblastoma cell extracts as a function of time of incubation in media containing 0.10 mM tyrosine. The cells were first maintained for 24 h in F-14 medium containing 0.12 mM phenylalanine, 2% dialysed foetal calf serum and no tyrosine. During the experiment the cells were maintained in the same medium with 0.10 mM tyrosine.

The increase in tyrosine hydroxylase activity in response to tyrosine in the medium could have been due to the synthesis of new enzyme molecules, or the activation of existing enzyme molecules. We have investigated this question by titrating NIE-115 extracts with specific anti-tyrosine hydroxylase γ -globulin. Some results are presented in Fig. 3, which show that inhibition of the higher level of tyrosine hydroxylase activity required proportionally more antibody. Extrapolation of the titration data to the abscissa gives equivalence points which indicate that 1.66 times as much antibody would be required to inhibit totally tyrosine hydroxylase activity of cells grown in 0.10 mM tyrosine as of those grown in 0.04 mM tyrosine.

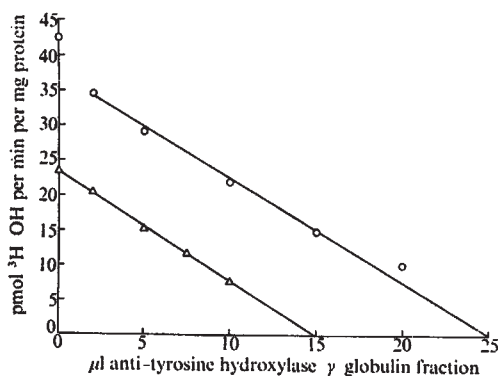


Fig. 3 Titration of tyrosine hydroxylase activity from NIE-115 neuroblastoma cell extracts with specific anti-tyrosine hydroxylase γ globulin. Extracts were prepared from cells maintained for 24 h in F-14 medium containing 0.12 mM phenylalanine, 2% dialysed foetal calf serum and 0.04 mM (Δ) or 0.10 mM ($\circ$) tyrosine. Each tube contained an equivalent volume of extract to be assayed (0.64 mg protein per tube from the 0.04 mM tyrosine dish extracts, and 0.72 mg protein per tube from the 0.10 mM tyrosine dish extracts). So that each sample would contain the same total amount of γ globulin, control γ -globulin fraction was added to bring them all to the same volume and the tubes containing no anti-tyrosine hydroxylase γ globulin contained only control γ globulin. The tubes were incubated at 25°C for 10 min, then at 0°C for 4 h and then assayed for tyrosine hydroxylase activity.

Extracts of the former cells contained 1.60 times as much enzyme activity as did extracts of the latter. Titration of extracts from cells grown in media containing other concentrations of tyrosine showed similar proportionality between enzyme activity and the amount of antibody necessary to achieve a given degree of inhibition. Thus, the increases in tyrosine hydroxylase activity are due to an increase in tyrosine hydroxylase molecules.

Increases in catechol and catecholamine synthesis in nerve cells in response to increasing levels of external tyrosine^{14,15} have been interpreted to imply that, at least under some conditions, uptake of tyrosine may be a rate-limiting step in catecholamine biosynthesis. Our results suggest that these observations also reflect the ability of tyrosine to increase the amount of cellular tyrosine hydroxylase. We have not yet determined whether the observed increase in tyrosine hydroxylase protein is due to increased synthesis or decreased degradation of the enzyme. Previous studies of increased enzyme activity in mammalian tissues caused by substrates have shown that the substrate can protect the enzyme against inactivation or degradation, for example, L-tryptophan on tryptophan oxygenase^{16,17}, and thymidine on thymidylate kinase¹⁸. Further studies are necessary to confirm or disprove the possibility that increased extracellular tyrosine elicits an increase in neuroblastoma tyrosine hydroxylase protein by protecting the enzyme against degradation.

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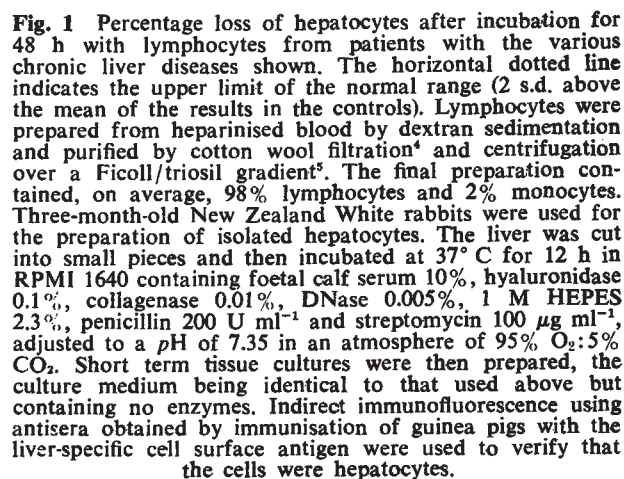
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Significant cytotoxicity was demonstrated in 20 (90%) of the patients with chronic active hepatitis, and was also present in two of the six patients with primary biliary cirrhosis (Fig. 1). In the 11 patients with other forms of chronic liver disease the results were similar to the controls, none showing significant cytotoxicity. Four of the 22 patients with chronic active hepatitis were untreated at the time of study and all showed significant cytotoxicity (56%, 70%, 89% and 84%). The remaining 18 cases were receiving various combinations of immunosuppressive drugs, and had shown improvement in biochemical liver function, but in only two did the result of the cytotoxicity test fall within the normal range. Serial studies in the four untreated patients, however, showed a significant reduction in the cytotoxicity following treatment with oral prednisone from a mean of 75–30% ($t=3.2$, $P<0.05$).



The blocking experiments clearly implicate sensitization to human LSP in chronic active hepatitis as the important factor in the production of the lymphocyte-mediated hepatocyte injury. Indeed, preliminary analysis has shown a good

correlation between sensitisation to LSP as detected by the leukocyte migration test and the degree of lymphocyte cytotoxicity to isolated hepatocytes. Such sensitisation might be initiated by structural changes in the membrane lipoprotein following virus or drug-induced liver damage, but little information is available concerning either this aspect of pathogenesis or the aberrations in immunological control systems which may determine persistence of autoimmunisation.

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Intramembraneous changes on cationophore-triggered exocytosis in *Paramecium*

SECRETORY vesicles ('trichocysts') of *Paramecium* contain proteins¹ which are discharged by exocytosis. In *P. aurelia* (mating type VIII), trichocysts are firmly attached to the cell membrane before the onset of exocytosis, and membrane-intercalated granules occur at these contact sites (Fig. 1); these granules are arranged in a regular pattern and have been described previously as 'b-' and 'c-type granules'. They are surrounded by a ring of 'a-type granules' which connect the cell membrane to the membrane of the alveolar sacs in the region where the latter are penetrated by the trichocysts^{2,3}. When the trichocysts are expelled, the cell membrane is penetrated within the ring of a-type granules and the trichocyst membrane fuses temporarily with the cell membrane². The present study takes advantage of several circumstances: (i) cells could be frozen without prefixation or cryoprotectants; (ii) exocytosis triggering involves only the final step of membrane fusion; (iii) the regular 'landmarks' in the plasmalemma serve to pinpoint intramembraneous changes occurring during exocytosis.

Exocytosis, monitored using the phase microscope, was triggered by enhancing transmembrane ion flux via cationophores, that is, small molecules incorporated spontaneously into membranes. Intramembraneous morphological changes were analysed by freeze-fracture. Ionophores used were X-537 A (Hoffman-LaRoche), which complexes reversibly with mono- and bivalent cations⁴, or A 23187 (E. Lilly), which complexes with bivalent cations only⁴. Exocytosis is connected in various cell types with Ca^{2+} influx⁵; the same ionophores also trigger exocytosis in

various other systems if Ca^{2+} is added⁶⁻⁸. In untreated *paramecia*, spontaneous trichocyst discharge is rarely observed. Addition of Ca^{2+} to *Paramecium* cultures does not provoke discharge in the absence of ionophores. The Ca^{2+} content of the medium (0.4 mM), determined by atomic absorption with a Jarrel Ash unit, is much higher than the concentration thought to occur within *Paramecium* cells (10^{-4} mM) (ref. 9) and is sufficient to trigger some exocytosis on addition of ionophores alone. Chelation of extracellular Ca^{2+} with 2.5 mM EDTA, which only gradually immobilised the animals, prevented trichocyst exocytosis on addition of 0.10 mM X-537 A. With appropriate concentrations of ionophore A 23187 (0.07 mM) and Ca^{2+} (17 mM), practically all *paramecia* discharged almost all their trichocysts within 20 min before gross morphological changes were detectable on the cells or their trichocysts, although the latter extend to several times their resting length during expulsion. Preliminary attempts to localise intracellular Ca^{2+} suggested that a Ca^{2+} shift into the trichocysts precedes exocytosis (H.P., unpublished). Trypan blue did not penetrate into a given cell before exocytosis of most trichocysts was completed, which excludes a bypass of Ca^{2+} influx through gross membrane leaks.

During freeze-fracture the plasmalemma splits along a central plane in *Paramecium*² as in several other membranes¹⁰. The fracture face attached to the cytoplasm is called A face: the complementary view of the split outer half represents the B face. Cell membranes of controls exhibit on their A face b- and c-type granules at the sites of trichocyst attachments; c-type granules are especially visible as ~800 Å clusters on the B face (Fig. 2a); the attachment sites of trichocysts are enclosed by a-type rings consisting of double (or occasionally single) rows of granules on the A face; corresponding holes occur on the B face. Since one cannot recognise on a replica of a freeze-cleaved cell membrane whether exocytosis has occurred,

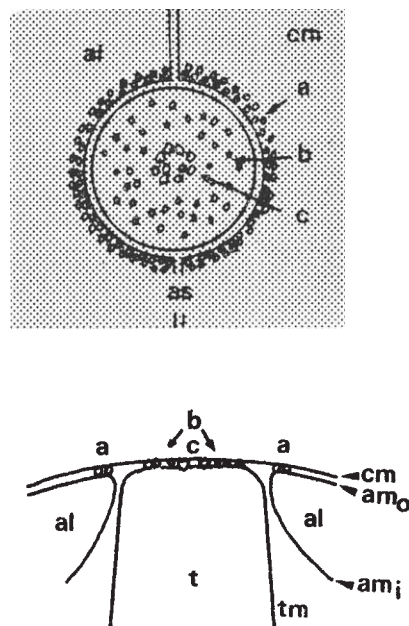


Fig. 1 a, A-type ring of membrane-intercalated granules, connecting cell membrane and alveolar membranes; al, alveolar cavities (interrupted at sites of trichocysts); am_i, (am_o) inner (outer) membrane of alveolar cavities; as, alveolar septa; b, diffuse aggregates of membrane-intercalated particles (located within a-type rings) at contact sites between cell membrane and trichocyst membrane; c, ~800 Å large patch of membrane-intercalated particles at the centre of the site of contact between trichocyst membrane and cell membrane (dotted); t, trichocyst; tm trichocyst membrane.

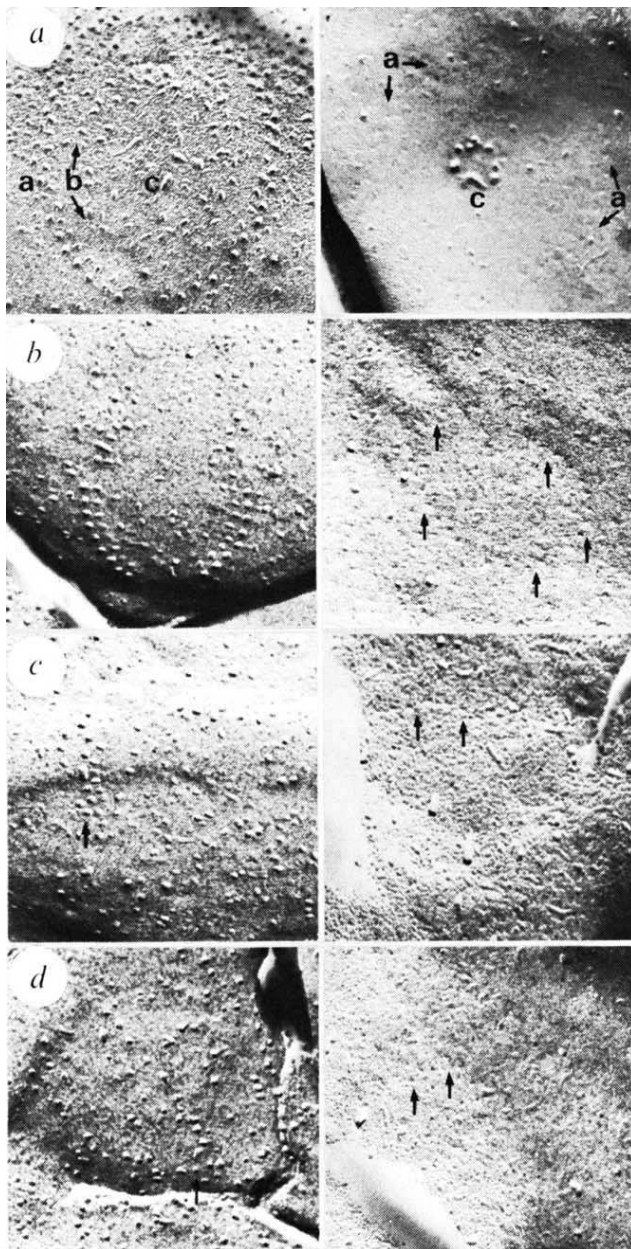


Fig. 2 A face (left) and B face (right) of the split cell membrane after freeze-cleaving. Controls (stage *a*) are compared with different stages of morphological changes after triggering exocytosis (stages *b-d*) obtained by adding 0.07 mM ionophore A 23187 and 17 mM Ca^{2+} for 15–18 min. Stages *b-d* may be encountered in close vicinity within one animal; c-type granules invariably disappeared after exocytosis. Stage *d* could be identified on the basis of the regular geometrical arrangement of trichocyst attachments on the body surface². All samples were frozen in Freon 12 without prefixation or cryoprotectants, cut at -100°C and shadowed with platinum-carbon in a Balzers BA 360 M freeze-etching unit. Micrographs are mounted with white shadows, the shadow casting direction is from bottom to top. a-Type rings and their remnants (arrows) appear as granules on the A face and as corresponding holes (faintly visible) on the B face. ($\times 100,000$.)

conditions were chosen, where complete triggering of exocytosis was achieved. After exocytosis a-type rings persisted in various degrees of decomposition on A and B faces (Fig. 2*b-d*, Table 1) and could therefore be used as 'landmarks' to locate the sites where exocytosis had taken place; furthermore, these sites are located at predictable points of the body surface². After exocytosis, c-type granules almost

invariably disappeared from the B face (Fig. 2*b-d*, right); b-type granules also seem to be dispersed, although they are arranged diffusely, even in controls. a-Type rings often persist as fragments, although the number of non-oriented particles seems greater in regions where a-type rings undergo decay (Fig. 2*c*, left). This suggests that granules are dispersed by lateral movement and that after exocytosis the cell membrane is resealed by lateral diffusion of membrane (lipid) constituents; this latter assumption is further supported by the observation that a-type rings are smaller in triggered cells than in controls (Table 1). Ultrathin sections show that the dispersion of a-type rings in the period following massive trichocyst discharge could be caused by a gradual detachment of the plasmalemma after exocytosis and membrane resealing have been completed. Thus, the most specific change related directly to exocytosis is the disappearance of c-type granule aggregates. Since these are present within most of the a-type rings in unstimulated, normal cells, their occurrence cannot be explained by membrane fusion during exocytosis^{11,12}; rather, in *Paramecium* they represent stable structural elements at the sites where trichocysts are attached permanently to the plasmalemma prior to the membrane fusion occurring during exocytosis.

The intracellular membrane-to-membrane attachment sites² of types a–c in normal *Paramecium* do not represent selective permeability sites which would be directly analogous to gap junctions¹³. This follows from studies with electron microscopic tracers such as $\text{La}(\text{OH})_3$; all membrane-to-membrane attachment sites of *Paramecium* are also impermeable to (2% w/v) microperoxidases (haemoligopeptides, molecular weight approximately 1,700–1,900), applied to living *Paramecium* over several days (H.P., unpublished). If, during spontaneous exocytosis, the membrane-intercalated particles function in analogy to ionophores (not resolvable in replicas) the particles would have to be capable of very rapid permeability changes specifically for Ca^{2+} . Other functional aspects of membrane-intercalated particles, such as the possible role of lipid constituents in membrane fusion¹⁴ and Ca^{2+} -dependent ATPases¹ possibly being involved in exocytosis also have to be considered.

Table 1 Morphological changes of a-type rings in freeze-fractured replicas after exocytosis*

Treatment	Decomposition stages		Size (nm)§	
	Stage†	Frequency‡ %	Small diameter	Large diameter
Controls	<i>a</i>	97	254 ± 43	313 ± 36
	<i>b-d</i>	3		
15–18 min 0.07 mM ionophore A 23187 + 17 mM Ca^{2+}	<i>a</i>	0		
	<i>b</i>	~28	195 ± 38	243 ± 29
	<i>c</i>	~26		
	<i>d</i>	~46		

*Samples were processed for freeze-cleaving as described in Fig. 2.

†Stages of decomposition (*a-d*) are as defined in Fig. 2.

‡Numbers of a-type rings counted were 59 for controls and 39 after exocytosis.

§Values $\pm$ s.d. Since trichocysts do not occur over the whole body surface, only those regions surrounded by intact or clearly visible remnants of a-type rings were evaluated; frequency values show that in such regions a-type rings are only rarely missing in controls; therefore, values determined for stage *d* after exocytosis are necessarily minimum estimates.

As discharge is very rapid, time sequence studies of morphological changes could not be performed. The occurrence of similar granular membrane specialisations has been reported not only in *Tetrahymena*^{11,12} and *Paramecium*² but also in connection with transendothelial movement of cytoplasmic vesicles^{15,16}. In contrast, no such intramembraneous differentiations were observed in various gland cells^{17–20}, stimulated or unstimulated. In these cases, membrane-to-membrane attachment might possibly have escaped detec-

tion, either because it is an ephemeral event or because of the pretreatments commonly used before freezing. Non-cryoprotected *Paramecium*, however, cationophore-mediated transmembraneous Ca^{2+} influx provokes trichocyst exocytosis, accompanied by specific changes of structural subunits preforms within the membrane portions involved.

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system (MFOS), an intracellular enzyme complex located within the membranes of the endoplasmic reticulum. This system is known to activate other halogenated hydrocarbons into hepatotoxins⁸. Therefore, we set up an experiment to determine whether the potential of VCM for liver injury was enhanced in animals pretreated with phenobarbital (PBT), an inducer of certain enzymes of the MFOS.

Male Holtzman rats (300 g) were exposed once to air, or air containing 0.5, 5 or 10% VCM for 6 h or to air alone or air containing 0.5 or 5% VCM for 6 h on each of 5 consecutive days in a dynamic inhalation chamber previously described⁹. VCM was obtained from Matheson Gas Corporation. Rats were given untreated drinking water regularly or they were pretreated with sodium phenobarbital (0.1%) in the drinking water beginning 7 d before exposure to VCM. This dose, which corresponds to 100 mg kg⁻¹ d⁻¹, is sufficient to double both cytochrome P-450 content and oxidative N-demethylase activity^{10,11}. Four experimental groups were defined: (1) exposed to air and not pretreated; (2) exposed to VCM and not pretreated; (3) exposed to air and pretreated with PBT; and (4) exposed to VCM and pretreated with PBT. Animals were killed 24 h after the single exposure, or immediately after the fifth. Blood was collected to measure serum enzyme activity indicative of liver damage⁹. The liver tissue was prepared for histological examination by perfusion fixation through the portal vein with buffered 1% glutaraldehyde. Selected blocks were post-fixed with OsO₄. Thin sections of Epon-embedded liver were examined by electron microscopy¹¹.

Table 1 Effect of phenobarbital pretreatment on serum enzyme activities 24 h after onset of a single 6-h VCM exposure

Exposure*	Pretreatment*			
	None		PBT	
	AKT‡	SDH§	AKT‡	SDH§
Air (5)	0.27±0.02	7.0±1.7	0.27±0.05	8.0±0.6
0.5% VCM† (3)	0.28±0.01	4.0±0.4	0.43±0.15	16.8±10.0
5% VCM† (2)	(0.17, 0.14)	(6.7, 5.6)	(3.41, 7.88)	(160, 256)
10% VCM (4)	0.42±0.10	25.1 9.8	—	—

*Numbers in parentheses are number of animals.

†VCM concentration verified by gas chromatography.

‡Alanine- α -ketoglutarate transaminase activity: mg pyruvate ml⁻¹ h⁻¹.

§Sorbitol dehydrogenase activity: units ml⁻¹ min⁻¹.

*Expressed as mean s.e. or as range.

The biochemical data from these experiments are shown in Table 1. In this table, both non-pretreated and PBT-pretreated animals exposed to air alone had no rise in levels of serum alanine- α -ketoglutarate transaminase (EC 2.6.1.2, AKT) or sorbitol dehydrogenase (SDH), another cytoplasmic liver enzyme whose appearance in serum correlates with liver injury^{12,13}. We have previously shown this enzyme to be highly specific for several types of liver injury¹⁴.

In non-pretreated groups, exposure to 0.5% or 5% VCM for a single 6-h period did not cause a substantial rise in either serum AKT or SDH activity. Only after exposure to 10% VCM was there a slight increase in either parameter of hepatotoxic response. In these animals, livers were histologically normal at the lower dose levels of VCM (Fig. 1a), and centrilobular hepatocellular vacuolisation was noted only in the group exposed to 10% VCM. During exposure to this higher dose level, animals appeared to be anaesthetised.

By contrast, PBT pretreatment for 7 d caused a marked enhancement of injury at the 5% level of exposure. Rats exposed to 0.5% were without apparent injury, determined biochemically. In the PBT-pretreated rats exposed to 5% VCM a clear morphological response was observed with striking vacuolisation of centrilobular parenchymal cells (Fig. 1b). There was also focal necrosis of mid-zonal parenchyma which became confluent towards the dorsal aspect of the liver. This vacuolated change corresponds to dilation of the rough endoplasmic reticulum as seen by electron microscopy. Smooth

Acute hepatic injury by vinyl chloride in rats pretreated with phenobarbital

LITTLE is known about the biological effects of vinyl chloride monomer (VCM) despite its wide industrial use. In 1973, thousands of workers were involved in producing, processing and moulding VCM into 15 billion pounds of its polymer (PVC)¹. Angiosarcoma, a rare liver cancer, has recently been linked to occupational exposure in man² and long term inhalation of low doses in animals³. Occupational vinyl chloride exposure has also been associated with increased incidence of hepatic cirrhosis, splenomegaly, decreased bromosulphalein clearance from blood and acro-osteolysis^{4,5}. Reports of acute hepatic injury after vinyl chloride inhalation are limited and the findings relatively nonspecific^{6,7}.

VCM, readily reactive in free radical-mediated industrial polymerisations, may interact with liver mixed function oxidase

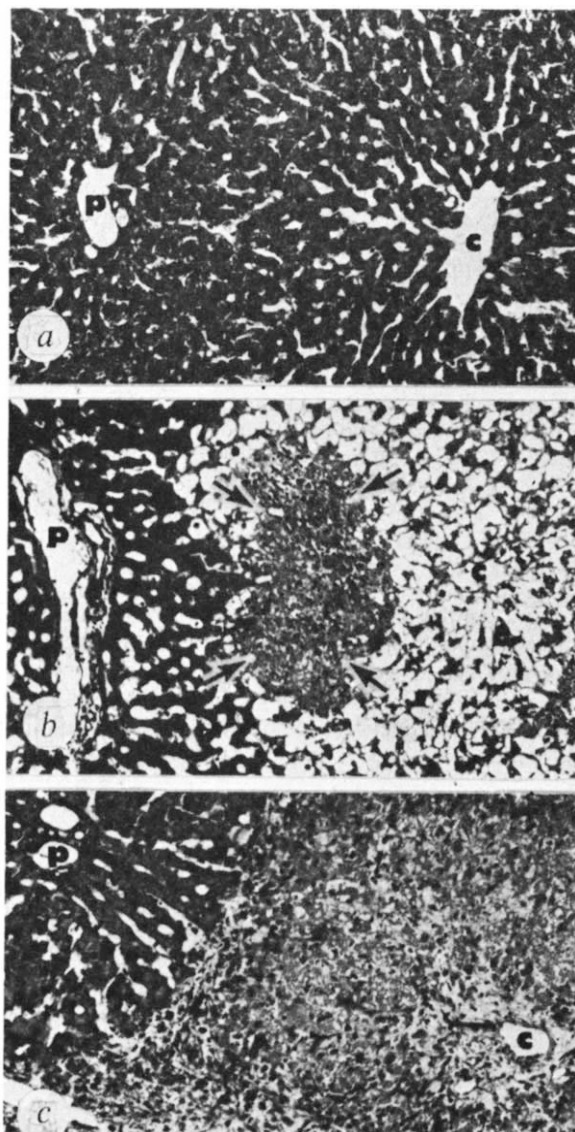


Fig. 1 Liver: portal areas (p) on left; central veins (c) on right. *a*, Non-pretreated rat 24 h after 6-h exposure to 5% VCM. *b*, PBT rat 24 h after 6-h exposure to 5% VCM. There is extensive vacuolisation of centrilobular parenchyma on right and a focus of midzonal necrosis at centre (arrows). *c*, PBT rat immediately after last of five 6-h exposures to 5% VCM at 24-h intervals. Necrotic centrilobular parenchyma on the right has been removed, remaining connective tissue stroma is infiltrated by macrophages. Vacuolated parenchymal cells are not seen. Stain: haematoxylin and eosin. $\times 150$.

endoplasmic reticulum in involved cells form tubular snarls with foci of increased electron opacity suggesting of membrane denaturation¹¹. In one of four PBT-pretreated animals exposed to 0.5% VCM, such vacuolisation, but not necrosis, was also observed. As noted previously, centrilobular vacuolisation was seen only in livers of non-pretreated rats exposed to 10% VCM.

The serum from PBT-pretreated rats killed after the last exposure to 5% VCM following 5 consecutive days of exposure did not contain raised activities of either serum AKT or SDH. This suggests that acute hepatotoxic injury was not cumulative, or that previous exposure to VCM blocked the biochemical response which followed re-exposure at short intervals¹⁵. Preliminary time course studies showed that increases in serum enzyme activities reached a maximum 24 h after the start of VCM exposure. Thus, injury from day 4 of exposure should have been shown on day 5 when rats were killed. Instead of a

biochemical lesion, Fig. 1c shows that livers of PBT-pretreated animals successively exposed to 5% VCM contained broad areas of parenchymal loss and stromal collapse most prominent towards the dorsal aspect of the liver. These bands correspond in distribution to the bands of confluent necrosis seen after a single exposure. Although these animals were killed 24 h after the fourth exposure, and immediately following the fifth, there was neither vacuolisation nor acute focal necrosis—findings indicative of recurrent acute injury. Results of the multiple 5% VCM exposure suggest that PBT-pretreated animals are protected against recurrent acute hepatocellular injury from daily VCM exposure by previous injury-inducing VCM exposure. Livers from non-pretreated animals similarly exposed up to 5% VCM on 5 consecutive days were indistinguishable from non-pretreated animals exposed to air.

The hepatotoxicity of vinyl chloride may be caused by a mechanism similar to that of other non-symmetrically chlorinated ethylenes such as trichloroethylene (TCE). Induction of MFOS enzymes by pretreatment with PBT or 3-methylcholanthrene enhances the hepatotoxicity of TCE¹⁶, possibly through an increased rate and/or altered route of metabolism¹⁷. Interestingly, after an initial exposure to TCE, the *in vivo* metabolism following subsequent exposures is not reduced, but the ratio of secondary metabolites found in the urine changes¹⁸. From our data, the initial exposure to VCM seems to protect against acute injury on re-exposure for at least 5 d. Perhaps, this initial exposure alters the pathway of activation of VCM in a manner not unlike that proposed for CCl₄ (ref. 15) which destroys MFOS enzymes involved in its metabolic activation to a hepatotoxin. Like TCE¹⁹, the metabolism of VCM may proceed through an epoxide intermediate. Activation to a reactive electrophile²⁰ is also in keeping with the tumorigenic potential of VCM. Experiments to define the interactions of VCM and the MFOS are underway.

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Application of β -D-glucuronides and glucose together suggests a new direction for cancer chemotherapy

KNOWN anti-cancer agents are unselective, attacking not only tumour cells but also other rapidly proliferating cells such as lymphocytes. Any difference between tumour and normal cells which might provide a basis for specific chemotherapy has therefore aroused interest. One such difference is the lower pH value of tumour tissue (about 6) compared with most normal tissue (about 7.3) in the presence of glucose^{1,2}.

The lysosomal enzyme β -glucuronidase has a pH optimum of 5.2 (ref. 3). We report here that the glucuronidase activity of tumours is selectively enhanced by previous administration of glucose, presumably because glucose selectively lowers the pH of tumour cells. Since glucuronides are biologically inactive^{4,5}, our observation would suggest the possibility of designing drugs which are preferentially activated in tumour cells.

Tumour cells (5×10^6), counted with a haemocytometer, were implanted subcutaneously in the neck of a male Hauben rat (6 weeks old). The spindle-shaped sarcoma was induced in 1968 using methylnitrosourea and repeatedly transplanted⁶. After 4 weeks the rat bearing the tumour was treated six times with glucose (2 g kg⁻¹) (50% in phosphate buffer pH 7.3) intraperitoneally at 40 min intervals. Two hours after commencing treatment, β -hydroxyquinoline- β -D-glucuronide (Light, Colnbrook, UK, 500 mg kg⁻¹, suspended in the glucose solution) was applied intraperitoneally. Six hours after beginning the experiment, the animal was killed, using ether. The tumour and surrounding muscle were removed, and 1 g of each tissue homogenised in 5 ml distilled water in a Potter homogeniser. The homogenates were then continually extracted with boiling ether for 2 h, the ether extracts dried over Na₂SO₄, evaporated to approximately 0.5 ml and the residue investigated by thin layer chromatography (TLC) (Silufol UV₂₅₄, Kavalier, CSSR). 8-Hydroxyquinoline was present in the tumour extract, but not in the muscle extract (R_F 0.39 in benzene/acetone (1:1) and 0.143 in benzene/methanol (95:5), identical with those of the control sample). 8-Hydroxyquinoline was detected using its UV-extinction property.

Table 1 Extinction of tissue homogenates at 480 nm

	Tumour	Muscle	
E* ₄₈₀	0.254	0.073	†Treated with 8-hydroxyquinoline- β -D-glucuronide and glucose
E* ₄₈₀	0.078	0.083	†Treated only with 8-hydroxyquinoline- β -D-glucuronide
E* ₄₈₀	0.084	0.082	†Untreated for control

E*₄₈₀ of a solution of 0.1 mg 8-hydroxyquinoline in 1 ml 2 N NaOH + 2 ml reagent = 0.375.

†All rats were approximately the same weight (200-220 g) and had tumours of approximately the same size (1 g).

A second tumour-bearing rat was similarly treated; the ether solution of the homogenates was totally evaporated, and the residue treated with 1 ml 2 N NaOH and 2 ml of a reagent containing diazotated *p*-nitroaniline⁷. The extinction was measured at 480 nm (Table 1).

A third rat was treated with 8-hydroxyquinoline- β -D-glucuronide (500 mg kg⁻¹, suspended in 2.5 ml phosphate buffer pH 7.3), but without glucose. The extracts were prepared as described above and investigated using TLC and photometry. 8-Hydroxyquinoline was not detected in the tumour or in the muscle extract (Table 1).

Two more rats, each bearing a neck tumour, were treated with 2-naphthyl- β -D-glucuronide sodium salt (330 mg kg⁻¹ Koch-Light Colnbrook), together with glucose (12 g kg⁻¹), and two other rats with the glucuronide without glucose. We could only detect 2-naphthol in the tumour extracts of the rats treated with both 2-naphthyl- β -D-glucuronide and glucose. Barely visible traces of 2-naphthol were detected in the other tissue homogenates. The extracts were investigated using TLC (silica gel), R_F 0.31 in benzene/methanol (95:5) and 0.61 in benzene-dioxan-acetic acid (90:25:4), identical to those of the control sample. Diazotated benzidine⁸ was used for detection.

The 2-naphthole content in the tumour tissue was also measured quantitatively. The residue of the extracts was dissolved in 2 ml ethanol and 0.02 ml of a solution containing diazotated benzidine (0.5 g benzidine in 0.14 ml concentrated HCl and 98.6 ml water mixed with 100 ml 10% NaNO₂ in water) were added. The extinction was measured at 415 nm against a solution of 0.02 ml reagent and 2 ml C₂H₅OH (Table 2).

Table 2 Extinction of tissue homogenates at 415 nm

	Tumour	Muscle	
E* ₄₁₅	0.372	0.036	†Treated with 2-naphthyl- β -D-glucuronide and glucose
E* ₄₁₅	0.028	0.031	†Treated only with 2-naphthyl- β -D-glucuronide
E* ₄₁₅	0.022	0.021	†Untreated for control

E*₄₁₅ of 0.2 mg 2-naphthol in 2 ml C₂H₅OH + 0.02 ml reagent is 0.231.

†All rats were approximately the same weight (200-220 g) and had tumours of approximately the same size (1 g).

Analogous results were obtained on repeating all experiments. We have therefore shown that previous glucose administration selectively enhances tumour glucuronidase activity. This has implications for the design of anti-cancer drugs^{9,10}; inactive glucuronides of active drugs, for example, the O-glucuronide of *p*-hydroxyaniline mustard¹¹, could be selectively activated in tumour tissue by this procedure. The synthesis of similar conjugates and investigations about its anti-cancer activity are in progress.

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Fatigue in frog single muscle fibres

WHEN a whole striated muscle is stimulated repetitively, its contractile force decreases and it is said to be fatigued¹⁻³. Fatigue does not occur in dystrophic muscles, and is characteristic of fast but not slow mammalian and amphibian muscle fibres^{4,5}. The complexity of the excitation-contraction process in fast muscle fibres has made it difficult to determine when fatigue sets in. Restoration of twitch tension of fatigued fibres by caffeine^{6,7} suggests that fatigue is a failure of calcium release, although the depletion, during prolonged contraction, of necessary energy-rich metabolites^{8,9} could be responsible for fatigue. Contraction can be uncoupled from calcium release and excitation by hypertonic solutions (refs 10-12 and unpublished results of S. R. Taylor, R. Rudel and J. R. Blinks). We have now found that hypertonic uncoupling prevents fatigue, so that fatigue must be linked to contraction. We have also found that single muscle fibres can recover after being fatigued by prolonged tetanisation.

Single, fast muscle fibres of the semitendinosus muscle of frogs (*Rana pipiens*) were used, rather than whole muscle, to avoid the rate-limited diffusion and the heterogeneous fibre

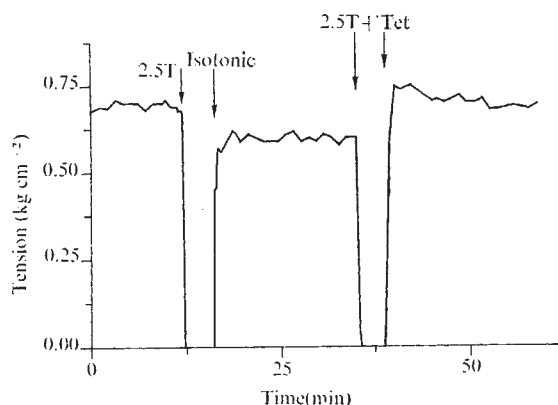


Fig. 2 Twitch tension blockage and recovery from immersion in Ringer made 2.5 times (T) isotonic by addition of Na_2SO_4 . Arrows indicate immersion period. During the second immersion the fibre was tetanically stimulated at 20 Hz for 120 s.

population in whole muscle^{13,14}. A fibre was dissected and mounted in a bath of flowing medium at 15°C. Tension was recorded by a RCA 5734 transducer tied to one end of the fibre, and was displayed on an oscilloscope screen and a paper chart recorder. Sarcomere length was measured by reading the interline distances of a laser diffraction pattern of the fibre¹⁵, and was adjusted to 2.3 μm . The fibre was stimulated by external platinum electrodes with 0.3 ms condenser discharges. Isometric twitch tension was between 0.7 and 1.2 kg cm^{-2} and tetanic tension at the beginning of tetanus varied from 1.6 to 2.2 kg cm^{-2} .

Figure 1a shows a typical response of a fibre to tetanic stimulation at 20 Hz for 120 s with interruptions of 11 s. During stimulation, tetanic tension decreased to 54% of its initial value in a graded rather than in an all-or-none fashion¹⁶. In the experiment, the twitch tension that followed the tetanus initially was lower than pre-tetanic control, increased transiently within 10 s and then decreased to close to zero at 10 min (Fig. 1b). It returned to the baseline during a prolonged recovery period of about 100 min (Fig. 1b).

The initial and short-lived increase in twitch tension after a tetanus represents post-tetanic potentiation, which is ascribed to a redistribution of calcium within the sarcoplasmic reticulum¹⁸. The increase is associated with prolongation of contraction time (time-to-peak tension) and of relaxation time (time-from-peak tension to baseline, Fig. 1b), also characteristic of post-tetanic potentiation¹⁶⁻¹⁸.

In contrast to the prolonged recovery of twitch height after tetanic stimulation in isotonic Ringer (Fig. 1b), recovery of twitch height after immersion in hypertonic Ringer (made 2.5 times (T) isotonic by adding sucrose or Na_2SO_4) was not affected by tetanic stimulation (Fig. 2). Fibres stimulated in hypertonic Ringer recover their basal twitch tension with a half time of 53 ± 15 s.e. ($n = 4$) s, compared with a half time of recovery in isotonic Ringer of 72 ± 7 s.e. ($n = 3$) min. As Fig. 2 shows the rate of recovery of twitch height after tetanic stimulation in hypertonic solution did not differ from the rate after simple immersion. The latter process depends on diffusion of water and hypertonic solute between bath and fibre¹³.

These results indicate that the decrement in twitch tension characteristic of fatigue in single muscle fibres is not due to an intrinsic failure of the calcium release mechanism in excitation-contraction coupling. Contraction is a process that consumes energy^{8,9}. Tetanic contraction fatigues the muscle fibre probably by depleting energy stores, which in turn may affect the release of calcium so that twitch tension is reduced.

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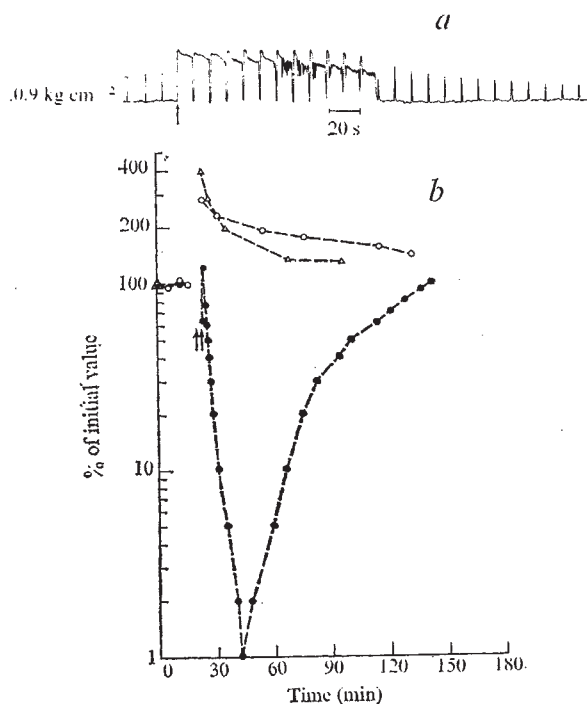


Fig. 1 a, Tension recording from a single muscle fibre tetanically stimulated at 20 Hz. Arrow indicates onset of stimulation. Recovery intervals correspond to 1 s without stimulation. Twitches are shown before and immediately after the tetanus. b, Logarithmic graph of peak tension (●), contraction time (○), and relaxation time (△) of twitches from the same experiment as in a. Values plotted are percentages of pre-tetanic levels. The absolute initial values of the parameters are respectively 1.14 kg cm^{-2} , 56 ms and 125 ms. The period between arrows indicates the tetanisation period.

science during immersion in hypertonic solutions. J. L. V. is a recipient of a postdoctoral fellowship from the Muscular Dystrophy Associations of America.

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Effect of dantrolene sodium on calcium movements in single muscle fibres

DANTROLENE sodium or 1-[5-(*p*-nitrophenyl) furfurylidene amino] hydantoin sodium hydrate (DaNa) is a long-acting drug with skeletal muscle relaxant properties which has recently seemed clinically useful in the treatment of some types of muscle hypertonia^{1,2}. DaNa does not affect neuromuscular transmission nor the electrical properties of skeletal muscle³ and it apparently involves the processes of excitation-contraction coupling^{1,3,4}. The mode of action of DaNa has not been studied before on single muscle fibres and here we provide direct evidence that DaNa interferes with the intracellular movements of calcium ions.

Rana temporaria (60 g) kept at 15°C and force fed twice a week with 1–1.5 g chopped beef were used. Single skeletal muscle fibres of the fast twitch type were isolated by microdissection from the dorsal part of the semitendinosus muscle. The fibres diameter 70–150 µm were kept in a standard phosphate Ringer solution⁵ at pH 7.2 and they maintained their physiological properties (including electrical threshold) for several hours. When dissected, the muscle fibre was transferred into a small (1.5 ml) Perspex chamber and mounted horizontally with one of its tendons hooked to the anode of a RCA 5734 mechano-electrical transducer tube. A home-made Perspex four-way tap allowed the thorough replacement of the chamber fluid within about 1 s. A continuous circulation system with appropriate bypasses maintained the temperature of the experimental solutions and of the chamber at 18°C. Electrical pulses of 0.2 ms duration and 1.2 times threshold were delivered

through a pair of bright platinum wires fixed 10 mm apart in the bottom of the chamber at right angles to the fibre. DaNa freshly dissolved in phosphate Ringer was applied at a concentration of 10 µM. This produced a mean depression of the twitch force by 58% (eight experiments) within 30 s and an additional 6% decrease during the next 10 min (Fig. 1a). The twitch contraction time and relaxation time were shortened. The action of DaNa was much more rapid for these single muscle fibres than for the whole muscles studied previously. Most of our data were collected within about 6 min of exposure DaNa. The effect of the drug was reversible on return to normal Ringer but a complete recovery of the twitch force took 30–60 min.

When the isometric twitch force was markedly reduced by DaNa, the tetanus force for a 1.5 s supramaximal stimulation at 100 s⁻¹ was not significantly reduced. The finding of a large reduction of the twitch with little, if any, change in tetanus tension bears out the earlier results on whole muscle^{1,4} and indicates that DaNa does not impair the maximum capacity of the muscle fibre to produce force. The recorded reduction of the twitch force by DaNa must therefore be related to an inhibition of some step in the process of mechanical activation, as the membrane spike generation is not impaired³.

A useful way to approach this problem is by recording single fibre contractions to known increases in the external potassium concentration whereby Hodgkin and Horowitz mechanical threshold⁶ can be estimated. Figure 1b shows a near maximum contraction of a fresh muscle fibre suddenly exposed to 50 mM

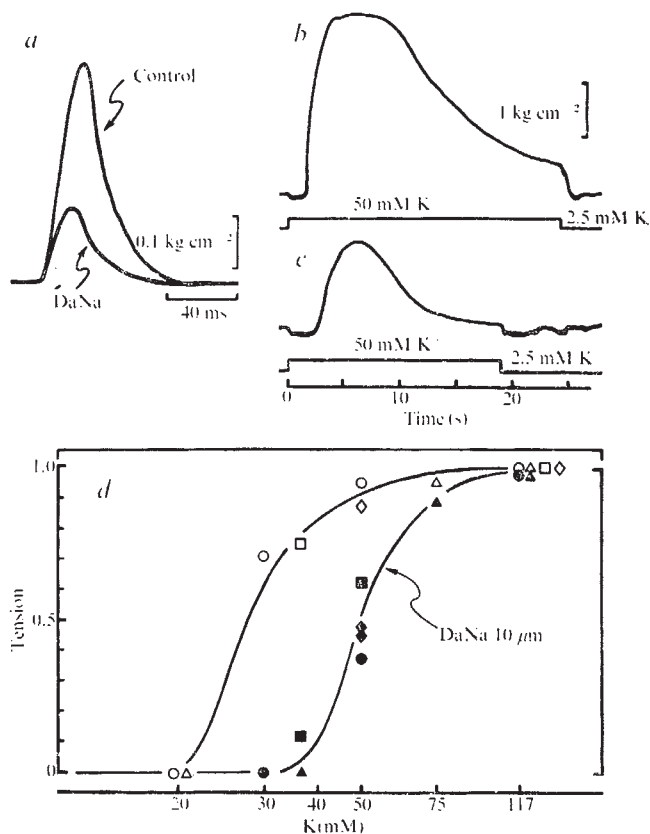


Fig. 1 Effect of DaNa on single muscle fibre. *a*, Superimposed isometric twitches elicited by supramaximal pulses before and in presence of DaNa; *b* and *c*, contractions elicited by a rapid increase in external K from 2.5 to 50 mM (see step), *b*, before and, *c*, in the presence of DaNa. The vertical calibration is expressed in kg force output per cm² cross sectional area of the muscle fibre. *d*, Relationship between peak contracture force and the increase of the external K (log scale). ○, □, △, ◇, before DaNa; ●, ■, ▲, ◆, in presence of 10 µM DaNa. Each different symbol corresponds to a different muscle fibre: ○ ● diameter 86 µm, maximum tension 3.1 kg cm⁻²; □ ■ diameter 74 µm, maximum tension 3.4 kg cm⁻²; △ ▲ diameter 98 µm, maximum tension 3.7 kg cm⁻²; ◇ ◆ diameter 118 µm, maximum tension 2.5 kg cm⁻². Temperature of solutions 18°C.

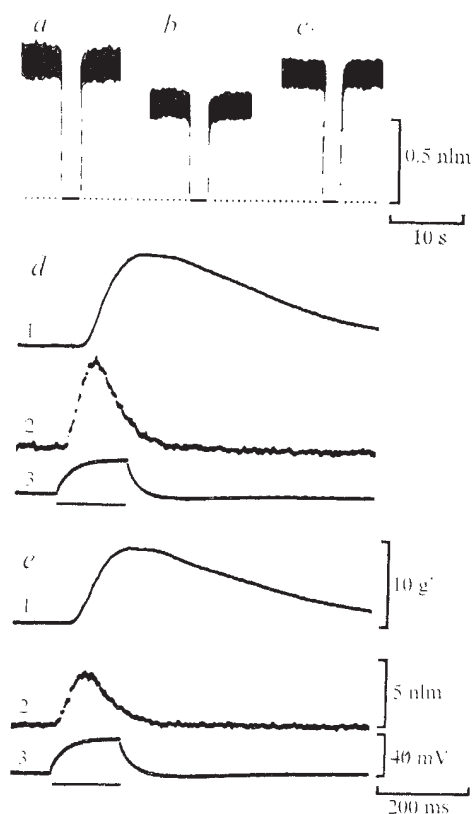


Fig. 2 *a-c*, Effect of 35 μ M DaNa in 0 Ca-1 mM ethyleneglycol-bis-(β -aminoethyl ether) N,N'-tetraacetic acid (EGTA) artificial seawater on the resting glow of an isolated barnacle muscle fibre injected with aequorin. The horizontal dotted line represents the 0 level. *a*, Resting glow after 3 min equilibration in 0 Ca-EGTA; *b*, decrease of the resting light emission after 30 s in DaNa; *c*, recovery in 0 Ca-EGTA seawater. Fibre diameter 1.1 mm; mean intracellular resting potential -55 mV. *d* and *e*, Result of applying a single electrical stimulation of 150 ms duration to a barnacle muscle fibre injected with aequorin, before, *d*, and after, *e*, 6 min exposure to DaNa 35 μ M in the external medium E. Trace 1, isometric tension; trace 2, calcium mediated light emission; trace 3, membrane response. Fibre diameter 1.8 mm. Mean intracellular resting potential -59 mV. Temperature of solutions 21° C.

K. Four min after bathing the fibre in 10 μ M DaNa (Fig. 1c) a second exposure to 50 mM K elicited a contraction which was reduced by more than 50%. The latency of this contraction was increased and its rate of rise reduced while the subsequent spontaneous relaxation during the maintained K exposure was quicker than in Fig. 1b. These effects of DaNa were reversible. The graph of Fig. 1, *d* shows similar data for different concentrations of K in four different muscle fibres. The peak force of each K contraction is expressed with respect to the maximum force recorded in the same fibre during exposure to 117 mM K. These maximum contractions were not significantly affected by 10 μ M DaNa in the present single fibre experiments and we think the reduction reported by others¹ must have been related to their use of whole muscle preparations⁶. The effect of lower K concentrations, however, was markedly reduced and the curve was shifted to the right by a factor of about 1.7. The mean threshold for K contractions was approximately 20 mM in untreated fibres and 35 mM K after 1 min in 10 μ M DaNa.

The observation that DaNa reduces both submaximal potassium contractions and twitch force without significantly affecting the maximum potassium contractions or the tetanus force could be explained by an inhibitory effect on some intracellular step in excitation-contraction coupling. To analyse this possibility, single muscle fibres from the barnacle *Balanus nubilus*⁷ were injected with aequorin, the calcium-sensitive

bioluminescent protein⁸. Several thousands of the jellyfish *Aequorea forskalea* were collected at Friday Harbor Laboratories during summer, 1973, and the extracted aequorin was purified in Brussels, using standard techniques⁹. Each barnacle muscle fibre of 1.0–2.0 mm diameter was cannulated with a Pyrex tube of 200 μ m diameter and mounted vertically in a light-tight black Perspex chamber⁸. The calcium-mediated light output was recorded on a Tektronix oscilloscope with an EMI 9635 photomultiplier tube. When the injected muscle fibre was placed in front of the photomultiplier, the rate of light emission related to the intracellular ionised calcium (resting glow) showed a rapid decrease during the first 30 min and then stabilised at a rather steady level as the $[Ca]_i$ is very low in the resting state and the rate of consumption of the injected aequorin is small. In these conditions, the replacement of the normal seawater by DaNa seawater was followed by a decrease of the resting glow to a lower level which depends on the drug concentration.

This reduction of the intracellular Ca concentration by DaNa could result either from a reduced resting Ca^{2+} influx, or from an increased resting Ca^{2+} efflux or from a direct action involving the intracellular Ca stores. To distinguish these possibilities, the aequorin-injected muscle fibres were tested in 0 Ca-1 mM EGTA artificial seawater, and DaNa 35 μ M still reversibly reduced the resting glow by about 20% (Fig. 2*a-c*) which suggests that the DaNa effect is not dependent on any reduction in resting Ca^{2+} influx. The second alternative could also be excluded in other experiments in which the leakage of ^{45}Ca into external seawater was estimated in barnacle muscle fibres loaded by an intracellular injection of 0.1–0.2 μ l ^{45}Ca in Tris buffer at pH 7.2 (ref. 10). The resting calcium efflux was actually decreased by 35 μ M DaNa which implies that the reduced intracellular calcium cannot result from an increased loss.

Therefore it would seem that DaNa acts primarily on the intracellular Ca storage sites to reduce the level of free sarcoplasmic calcium. This third alternative is indeed supported by studies of the calcium transient recorded during single contractions of barnacle fibres injected with aequorin. The fibre was stimulated at intervals of at least 20 s through an intracellular silver wire with a constant current pulse of 150 ms while the membrane potential was recorded with another intracellular platinum electrode connected to a high input impedance amplifier⁸. Although the actual membrane depolarisations thus elicited were identical before and after DaNa (third trace in Fig. 2*d* and *e*), the mechanical force (first trace) produced by the stimulated muscle fibre was reduced by 20% after DaNa 35 μ M while the transient increase in aequorin luminescence (second trace) decreased by about 30% in this example. These results were regularly recorded in the six different barnacle muscle fibres studied, each one being tested with various combinations of stimulus durations and intensities.

In conclusion, DaNa depresses the mechanical force output of single muscle fibres by an inhibitory effect involving the calcium movements at intracellular storage sites, both at rest and during electromechanical activation.

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Simultaneity, occlusion and the sodium pump

GARRAHAN and Garay^{1,2} have shown that for the sodium pump of the human red blood cell, the half-saturation concentration for transport of a particular cation from one face of the membrane is not affected by the type or concentration of cation at the opposite face. This finding is consistent with previous observations on the squid axon³ and the sheep red blood cell⁴. The simplest explanation of these results, according to Garrahan and Garay², is that the pump binding sites are at equilibrium with cation and that transport occurs only when cations are bound simultaneously at both faces of the membrane. Their view is that previously proposed "sequential" models⁵ must be rejected and replaced by models in which cation binding sites are present simultaneously on both sides of the membrane. In addition, they show that the transport data are sufficient to reject a model (Fig. 1c) if the pump exists for a substantial time in a conformation in which bound cations are occluded. Yet there is biochemical evidence⁶ that, at least at 0° C, the pump does nonetheless exist in an occluded state for an appreciable time. Here we show that this apparent paradox is resolved by the tetrameric internal transfer model⁷ of Fig. 1d.

The simplest 'simultaneous' models are shown schematically in Fig. 1; the applicable steady state kinetic schemes and their solutions are shown in Fig. 2 (for models *a* and *d* of Fig. 1) and in Fig. 3 (for models *b* and *c*). We first consider the model of Fig. 1a. Here, a binding site which is sodium-selective in one conformation moves physically across the membrane and becomes potassium-selective in the other conformation⁸. In the kinetic solution (see Fig. 2), the half-saturation concentrations for transport, being simply the constants K_s and K_p , are independent of the *trans* cation as required by the experimental data^{1–4}. This model makes no provision, however, for an occluded form and in Fig. 1b we have generalised to allow for this possibility. But in doing so we see from the kinetic solution (Fig. 3) that the half-saturation concentrations are no longer K_s and K_p but are variables which depend on the *trans* concentration of cation. Only if the concentration of the occluded form is a negligible fraction of the doubly-bound open forms will the experimental transport observations be satisfied; this conclusion is valid even for a more general model with a succession of occluded forms.

Figure 1c and *d* show two types of internal transfer model^{9,10}. A basic feature of such models is the presence of occluded forms. During each conformation change, transportable substrate is moved only part of the way across the membrane, since the binding sites themselves move only between a bathing solution and an internal cavity. Within this internal cavity, substrate can move from one binding site to the complementary site. During a second conformation change, substrate can then move out of the internal cavity and into the opposite bathing solution. Garrahan and Garay² have shown that the dimer model (Fig. 1c) yields the kinetic solution of Fig. 3, so that once again the concentration of the

occluded form would have to be insignificant. Finally, for the tetrameric internal transfer model (Fig. 1d), the simple kinetic scheme and solution of Fig. 2 are appropriate, because the steps labelled 'transport' now represent the release of substrate which entered the internal cavity during the preceding conformation change.

In summary, of the four 'simultaneous' models of Fig. 1, only the tetrameric internal transfer model both accounts for the experimental transport observations^{1–4} and allows for the existence of an occluded form during an appreciable fraction of the transport cycle. Thus, to distinguish between these models, one need only determine whether or not the fully-bound forms of the pump exist to an appreciable extent in occluded states.

What appears to be an occluded form of the pump from guinea pig kidney has been detected by Post *et al.*⁶. From their data it is possible to obtain a rough estimate of the

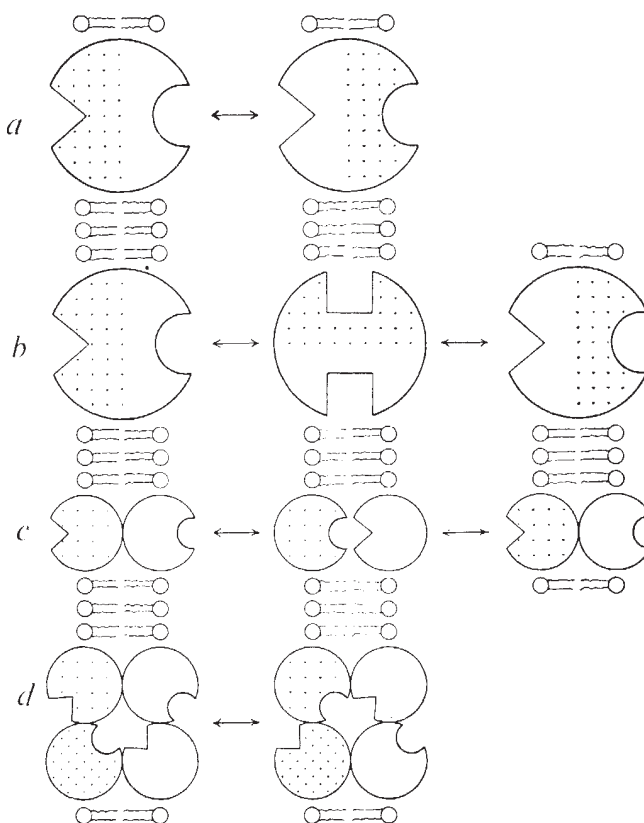


Fig. 1 Simple 'simultaneous' models for active transport. For each model, transport of substrates (not shown) occurs as a result of transitions between the depicted conformations of the transport protein. The substrate binding sites are the sharp, round, and square indentations; the sharp sites are specific for one substrate, the rounded for the second substrate, while the square sites are of undefined specificity. Notice the changes in specificity of a given site from one conformation to another. Only those portions of the transport proteins immediately adjacent to these binding sites are shown; stippling is used to distinguish these regions during the conformation changes. For example, although the two conformations in model *a* are functionally equivalent, the stippling indicates that they are not physically equivalent, but that the sites have moved completely across the membrane and switched their specificities during the conformation change. Notice that models *b–d* possess sites in occluded locations, while *c* and *d* are in addition internal transfer models^{9,10}. Two postulates made for all models are that the binding sites are in equilibrium with the substrates and that conformation changes occur only when all of these sites are fully occupied. (Although each site may require the binding of more than one substrate particle to be completely occupied, the analysis is simpler and the essential points are unaffected by taking the case of only one particle per site.) The drawings are of course wholly schematic, describing the formal basis of the transport events but not the detailed structural features of the transport proteins.

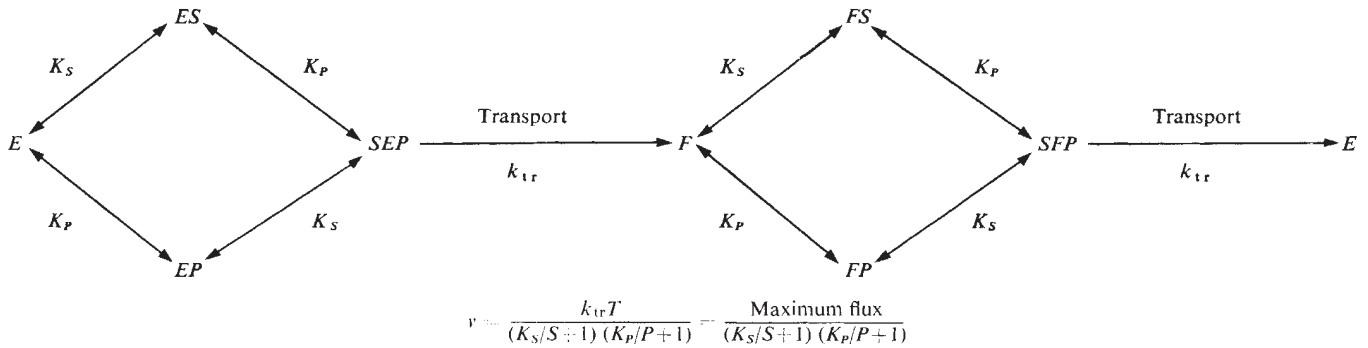


Fig. 2 Kinetic scheme and steady-state solution for the transport models of both Fig. 1a and d. *S* and *P* refer to transportable substrates and their concentrations in the left-hand and right-hand bathing solutions, respectively. *E* and *F* refer to the two conformations when the outward-facing binding sites are unoccupied, whereas *ES*, *EP*, *SEP*, *FS*, *FP*, and *SFP* refer to these conformations when the outward-facing sites are occupied by *S* and/or *P*. K_S and K_P are the equilibrium dissociation constants. The rate constant for the transport event is k_{tr} and T is the total concentration of pump. The unidirectional flux of *S* in exchange for *P*, which of course equals the corresponding flux of *P* in exchange for *S*, is given by v ; the solution for v is the same as that previously obtained by Garrahan and Garay² for the model of Fig. 1a. Although it is here assumed that each bathing solution contains only a single type of substrate, introduction of competing substrates results in the same expression for v but with the values of K_S and K_P effectively increased. Notice that for the model of Fig. 1d all forms of the pump contain in addition two substrate particles in an occluded state.

lifetime of this form at 0°C. Under the conditions of their Fig. 10, they consider the rate of rephosphorylation of the pump enzyme to be largely determined by the rate of disappearance of this occluded form. The maximum measured rate was obtained with high levels of ATP and had a half-time of about 3 s, from which (on dividing by $\ln 2$) the average lifetime of the occluded form would be some 4–5 s. But perhaps rephosphorylation occurs only after the occluded form disappears, so that this is an overestimate. Post *et al.*¹¹ found, however, that the phosphorylation reaction by itself is complete in less than 1 s at 0°C. Thus it seems that the occluded form exists for some 3–5 s at 0°C.

To determine for what fraction of the total time the occluded form exists, it is necessary to know the average time for a complete pump cycle. This information can be obtained from the experiments of Kanazawa *et al.*¹² with the rabbit brain enzyme at 0°C. Under the conditions of their Fig. 3 which are comparable to those of Post *et al.*⁶ the K-stimulated (Na+Mg)-ATPase activity was about 0.11 $\mu\text{mol P}_i \text{ h}^{-1}$ per mg protein. The maximum incorporation of ^{32}P from ^{32}P -ATP (Table 2 of ref. 12) was 1.35×10^{-4} μmol per mg protein, yielding an average cycle time of 4.4 s. This time may be marginally underestimated due to sub-maximal incorporation of ^{32}P . Using ouabain binding and the ionic binding of ATP to estimate pump concentrations¹³ one obtains turnover

numbers at 37°C averaging some 9,000 min^{-1} whereas from Fig. 3 and Table 2 of ref. 12 one calculates a value of 11,000 min^{-1} ; a correction factor of 1.2 might, therefore, be necessary, giving an average cycle time of up to 5.5 s. Overall, taking the range 3–5 s for the lifetime of the occluded form and 4–5.5 s for the cycle time, it seems that at 0°C the sodium pump spends a very substantial fraction of its time in an occluded form. (This time is presumably spent waiting for the rare thermal event of having sufficient energy to overcome the activation barrier between conformations. The time required for the conformation change itself may be rather short (see, for example, the half time¹⁴ of 14 ms for a photon-induced conformation change of rhodopsin in frog retina at 0°C).)

It would thus seem that the first three models of Fig. 1 must be rejected. We are hesitant, however, to adopt this conclusion unequivocally until either the transport observations are confirmed at 0°C or the occluded form is demonstrated at body temperature, or both these findings are confirmed at some intermediate temperature. Nonetheless, there is some indication that the effect at 0°C of ATP binding to a low affinity site and accelerating the release of an occluded form⁶ occurs also at 37°C. At this temperature, binding of ATP to a low affinity site accelerates both the (Na+K)-ATPase activity of the bovine brain enzyme¹⁵ and the K:K exchange across human red blood cell membranes¹⁶.

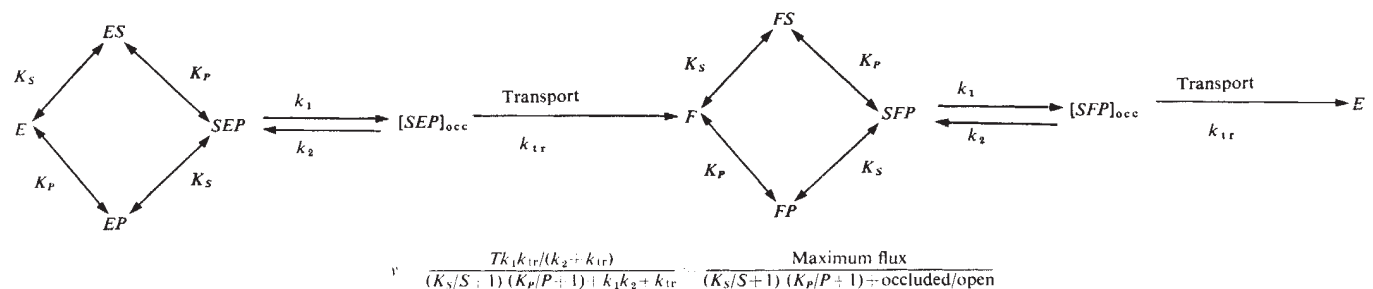


Fig. 3 Kinetic scheme and steady-state solution for the transport models of both Fig. 1b and Fig. 1c. Symbols and comments are as in Fig. 2, with the following additions: k_1 and k_2 are rate constants for reversible non-transporting transitions between open and occluded conformations; $[\text{SEP}]_{\text{occ}}$ and $[\text{SFP}]_{\text{occ}}$ refer to the occluded doubly bound forms of *E* and *F*, respectively; and the ratio 'occluded/open' = $[\text{SEP}]_{\text{occ}}/\text{SEP} = [\text{SFP}]_{\text{occ}}/\text{SFP}$. The kinetic solution is that of Garrahan and Garay².

It is encouraging that powerful rejection criteria can be devised on the basis of simultaneity and occlusion. With such criteria one can narrow down the number of possible models for active transport and so reach a clearer understanding of the molecular mechanisms involved.

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Lateral interaction between neural channels sensitive to velocity in the human visual system

MACKEY¹ has made use of the phenomenon of simultaneous contrast to provide evidence for the existence in the human visual system of neural channels that are sensitive to the density of visual texture. Using the same phenomenon, we have provided comparable evidence for the existence of lateral interaction between channels sensitive to velocity. That such channels do exist is suggested by the preliminary psychophysical observations of Pantle and Sekuler², who demonstrated a luminance threshold elevation for moving contours that is limited to a range of values around the velocity of the adapting contour.

A 10×6 matrix of dots was generated on the CRT display of a PDP-12 laboratory computer. The ten symbols in each row were programmed to move at a constant velocity in a west-east direction, giving the impression of a continuous stream of dots drifting across a window. A sharp discontinuity (or border) was generated by having the upper set of three rows move at a slower speed than the lower set. Viewed from a distance of 0.8 m the rows and columns of the matrix subtended visual angles of 3° and 1.88° respectively.

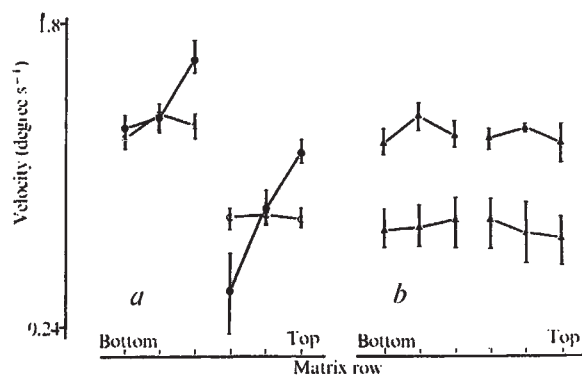


Fig. 1 Perceived velocity of each of the six rows in the matrix of dots, with each point representing the average of three observations. The vertical bars delineate the range of values spanned by the two extreme observations in each case. *a*, ●, The perceived velocity profile obtained when velocities of 0.30 degree s⁻¹ and 0.6 degree s⁻¹ were assigned, respectively, to the upper and lower rows in the matrix. The profile adds confirmation to the illusion reported by several observers; ○, with the fast and slow moving rows presented to separate eyes (the left and right respectively) simultaneous contrast does not occur. *b*, Profiles obtained in control trials in which all six rows were assigned the same velocity, being in the one case 0.6 degree s⁻¹ (▲) and in the other, 0.3 degree s⁻¹ (△).

With velocities fixed at 0.3 degree s⁻¹ and 0.6 degree s⁻¹, for the upper and lower rows respectively, the authors and several colleagues have experienced an illusion that is consistent with lateral interaction effects. More specifically, the difference in velocity of the rows adjacent to the discontinuity appeared enhanced when compared with the corresponding difference between more outlying rows (for example, the top compared with the bottom row). Perhaps counter-intuitively, the regular columnar organisation seemed to assist in the appreciation of the illusion; the apparent differences in velocity being translated into noticeable deviations from this regularity.

In order to determine more precisely the perceived velocity 'profile' of the matrix, a probe, or comparison row of eight dots was added to one side of the display. Two parameters of this row were made variable: (i) its vertical position could be programmed to coincide with any one of the six rows in the matrix, and (ii) its velocity could be altered by repositioning a smooth lever. By matching the comparison row to each of the six rows of the matrix, an estimate of the perceived velocity profile could be obtained.

One of the authors (P.W.) overcame the initial difficulties that were experienced in trying to both observe the illusion and match the velocity of a selected row and provided the profile illustrated in Fig. 1*a*. For comparison, the same procedure was followed whilst the dots in all six rows moved with the same velocity, being in the one case 0.6 degree s⁻¹ and in the other 0.3 degree s⁻¹ (Fig. 1*b*). A comparison of the different profiles highlights the simultaneous contrast character of the illusion. Furthermore, incorporated in Fig. 1*a* are the results from a condition involving the presentation of the 'fast' and 'slow' moving rows to separate eyes (the left and right respectively). That the illusion does not occur in this condition is consistent with MacKay's results with texture density, and suggests that the lateral inhibitory processes responsible for the illusion reside in the monocular systems, before the point of binocular fusion.

To summarise, the demonstration suggests that the human visual system incorporates channels that are sensitive to velocity and that these channels interact to enhance any discontinuities in this parameter. That there are such channels in the visual systems of cat and monkey is suggested by the discovery of single cells that respond differentially to the speed of a moving stimulus. Such units have appeared in the superior colliculus,

suprasylvian gyrus, and visual area 19 of cat, and in the primary visual cortex of monkey³. The present data may be taken to suggest that with velocity, as with orientation⁴, lateral inhibition effects will be observed at this single cell level.

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Electrophysiology of mammalian spinal cord *in vitro*

MAMMALIAN spinal cord has often been used in electrophysiological studies where the experiments were carried out exclusively on the spinal cord *in situ*¹. An obvious disadvantage of such experiments is that the concentrations of ions or drugs studied cannot be controlled precisely in the extracellular medium. To overcome that disadvantage, we have developed an isolated spinal cord preparation of the newborn rat.

Wistar rats aged from 0–7 d were used. Under ether anaesthesia, the spinal cord was isolated, hemisected sagittally and placed in a bath perfused with Krebs solution equilibrated with 95% O₂ and 5% CO₂. The temperature in the bath was kept at 27 ± 1° C. Changes of the membrane potentials of the spinal motoneurons were followed by recording from the ventral root with an extracellular electrode used in two ways. In the first, the ventral root (L3–L5) was led out through a vaseline-sealed slit in the lateral wall of the bath into a paraffin compartment where the root was placed on a Ag–AgCl electrode, which in turn was connected to a preamplifier and an oscilloscope or pen recorder. The bath was grounded through a reference calomel electrode. In the second method, the ventral root was introduced into a glass suction electrode (Fig. 1a). The portion of the ventral root between the spinal cord surface and the orifice of the suction electrode was covered by a mixture of liquid paraffin and vaseline contained in the glass

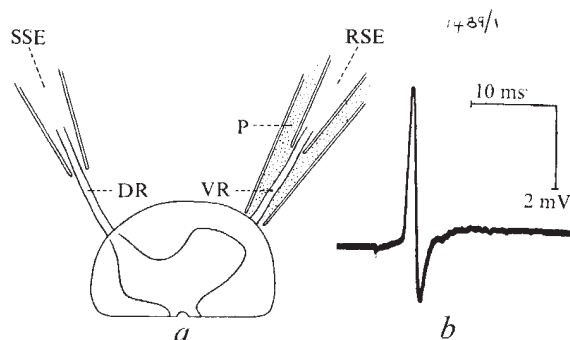


Fig. 1 a, Experimental arrangement. The recording suction electrode (RSE) was connected through a Ag–AgCl electrode to a preamplifier. SS, Stimulating suction electrode; VR, ventral root; DR, dorsal root; P, liquid paraffin. b, Mono- and polysynaptic reflexes induced by a supramaximal volley in L5 dorsal root and recorded from the corresponding ventral root. Positivity upwards.

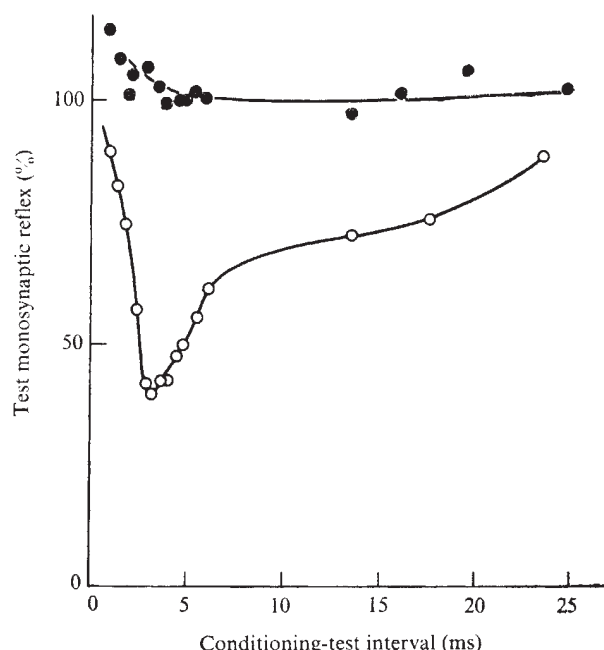


Fig. 2 Postsynaptic inhibition and the effect of Cl deficiency. Test reflex response was induced by a supramaximal volley in L5 dorsal root and was recorded from the corresponding ventral root. Single supramaximal conditioning volley was delivered to L4 dorsal root. Graph shows the amplitudes of the test monosynaptic reflexes expressed as percentages of the unconditioned control and plotted against the conditioning-test intervals, ○, in normal Krebs solution; ●, in Cl-deficient medium, where 9/10 of Cl ions were replaced by equimolar methylsulphate and methanesulphonate.

cuff. The cuff surrounded the inner suction electrode and was slightly pressed against the surface of the spinal cord. A quite stable d.c. recording was possible in these conditions.

Stimulation of the dorsal root (L3–L5) induced an early sharp spike followed by the later asynchronous waves recorded from the corresponding ventral root (Fig. 1b). The early spike was interpreted as the monosynaptic reflex because its wave form resembled that of monosynaptic reflex observed in the *in situ* experiments² and because the delay time of the spike (about 3 ms in the experiment shown in Fig. 1b) could be explained for the most part by the conduction time in the afferent and efferent pathways³. The preparation was quite stable and a good reflex response could be recorded for at least several hours.

Repetitive stimulation of the dorsal root at 0.3–1 Hz caused a marked decline of the sizes of mono- and polysynaptic reflexes. After tetanic stimulation, a slight increase in the amplitude of monosynaptic reflex (about 130% of the control) was observed. In the experiment illustrated in Fig. 2, L5 dorsal root was stimulated at 0.1 Hz and the reflex response was recorded from the corresponding ventral root. Conditioning stimulation of the adjacent dorsal root (L4), which by itself produced only very slight potential changes in the L5 ventral root, exerted during the following period of several milliseconds a remarkable inhibitory effect on the monosynaptic reflex. Judging from the fact that this inhibition displayed a relatively fast time course and was readily blocked by strychnine (5×10^{-6} g ml⁻¹), we conclude that it is the postsynaptic inhibition¹. When the concentration of chloride ions in the external medium was reduced to one-tenth of the normal, the postsynaptic inhibition was completely abolished (Fig. 2). This strongly indicates that the permeability increase to chloride ions is of predominant importance in this type of inhibition (compare with refs 1, 4 and 5).

The isolated spinal cord preparation described here seems to provide many possibilities for electrophysiological, pharmacological and developmental studies of the spinal cord.

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Excitatory action of hypothalamic substance P on spinal motoneurons of newborn rats

It is generally believed that the primary afferent fibres constituting the spinal dorsal roots, form excitatory synapses with the neurones in the spinal cord and the brain stem¹. Several substances have been considered as the neurotransmitters of the primary afferent neurones¹⁻⁵. We have found that an undecapeptide, substance P, which has been isolated from bovine hypothalamus^{6,7}, exists in the dorsal root of bovine spinal nerve in an amount 10-30 times larger than that in the ventral root^{8,9}, and that this peptide exerts a potent excitatory action on the spinal motoneurons of the frog^{8,10}.

These findings led us to postulate a hypothesis that hypothalamic substance P is a neurotransmitter of the primary afferent neurones and, to explore this hypothesis further, we examined the effect of hypothalamic substance P on the neurones of the mammalian spinal cord. The isolated spinal cord preparation of the newborn rat is suitable for this purpose because substances can be applied by adding to the perfusion

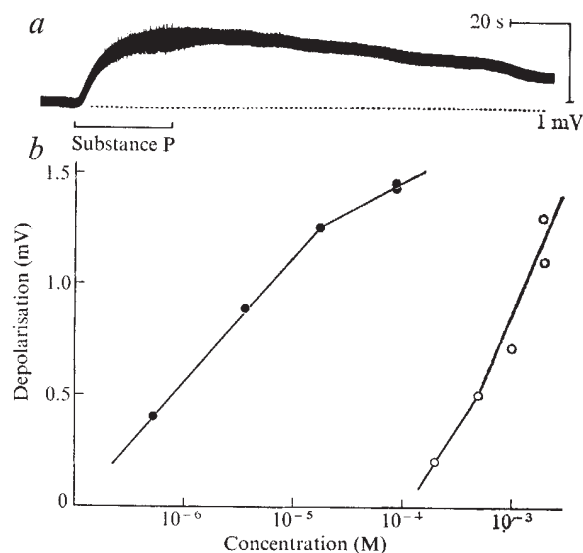


Fig. 1 *a*, The potential recorded from the L4 ventral root of an isolated spinal cord of a newborn rat. Hypothalamic substance P (9×10^{-5} M) was bath-applied during the period indicated. Depolarisation is upwards; dotted line indicates the control level. *b*, Dose-response curves of hypothalamic substance P (●) and L-glutamate (○), obtained in another preparation. Ordinate, amplitude of the induced depolarisation recorded from L4 ventral root; abscissa, molar concentration of the applied drugs on a logarithmic scale.

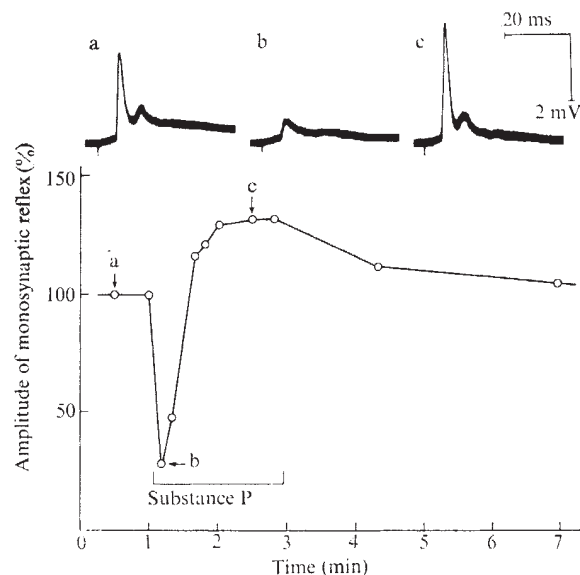


Fig. 2 Effect of hypothalamic substance P (3×10^{-5} M) on the spinal reflex. L4 dorsal root was stimulated supramaximally at 0.1 Hz and the reflex responses were recorded from the corresponding ventral root. Graph shows the amplitude of the monosynaptic reflex plotted over time. Substance P was bath-applied during the period indicated in the graph. Upper oscillographic traces show sample records at the times indicated by arrows in the lower graph.

fluid in controlled concentrations, which enables us to make an exact estimate of the relative potencies. The method of recording the electrical activities generated in the motoneurons was the same as described in the accompanying paper¹¹.

When Krebs solution in the bath was replaced by the solution containing synthetic hypothalamic substance P, a depolarisation accompanied by the spike discharges of spinal motoneurons was recorded from the ventral root (Fig. 1*a*). After the bath was washed out with Krebs solution, the recovery took place with a slow time course of a few minutes. L-Glutamic acid of relatively high concentrations induced similar depolarisation and spike discharges but the time course of the response was faster than that of substance P. In Fig. 1*b*, the relationship between log molar concentration and the induced depolarisation was plotted for both hypothalamic substance P and L-glutamate. From the comparison of the dose-response curves at the level of 1 mV potential change, the potency of the depolarising action of substance P was estimated to be about 200 times higher than that of L-glutamate which is regarded as a leading candidate for the excitatory transmitter in the spinal cord².

To find out whether hypothalamic substance P causes the depolarisation by directly acting on the motoneurons or by a trans-synaptic mechanism, the effect of the peptide was re-examined after the intraspinal synaptic transmission was blocked in a low-Ca and high-Mg solution. When the preparation was soaked in a modified Krebs solution containing 0.4 mM Ca and 7 mM Mg, the ventral root reflex evoked by dorsal root stimulation was completely abolished. At this stage the depolarisation induced by substance P was as large as that in normal Krebs solution, suggesting that the peptide has a direct excitatory action on the spinal motoneurons¹⁰.

Figure 2 shows the effect of hypothalamic substance P on the spinal reflex. First, the peptide induced a transient inhibition followed by a potentiation of the monosynaptic reflex. The first inhibitory effect of substance P may be due to the activation of the GABA-operated inhibitory mechanism because this inhibition was almost completely abolished after the treatment of the preparation with picrotoxin (10^{-5} g ml⁻¹). The second facilitatory effect, on the other hand, can be explained by the depolarisation of the motoneurons.

Thus the present results give further support to the hypothesis that hypothalamic substance P may serve as an excitatory transmitter of the primary afferent neurones in the spinal cord.

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A new type of hereditary persistence of foetal haemoglobin: is a diffusible factor regulating γ -chain synthesis?

PERSISTENCE of foetal haemoglobin synthesis into adult life produces a condition known as hereditary persistence of foetal haemoglobin (HPFH). Different types of HPFH have been described¹ but the precise molecular mechanism(s) responsible for them has not been clarified. Several genetic hypotheses have been proposed, of which the most widely accepted is that of a deletion in the $\delta\beta$ complex; this would agree with the fact that both the gene(s) for HPFH (so far described) and the β -structural locus behave as alleles. We have studied a new type of HPFH in a negro family, the gene for which does not behave as an allele of the $\delta\beta$ complex, and have therefore proposed a genetic interpretation for this condition.

The index case was found during the course of a systematic re-examination of patients previously diagnosed on clinical grounds as cases of sickle-cell anaemia. The pedigree and the haematological data of the family are shown in Fig. 1 and Table 1. The pedigree shows that a β^s , a β^{thal} and a gene for the persistence of foetal haemoglobin at approximately 5% level, are segregating in this family. While β^s and β^{thal} behave as usual (they are found separately in I₁ and I₂, together in II₁,

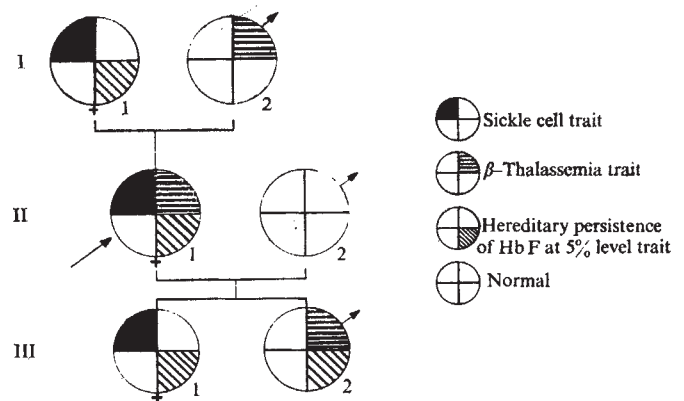


Fig. 1 Pedigree of the family AMR. The arrow indicates the proposita.

and segregate again in III₁ and III₂), the mode of transmission of the gene for HPFH is very strange. In fact, II₁ who has the responsible gene in a single dose (her father has normal levels of foetal haemoglobin (HbF)), transmits it together with the β^s (both genes received from I₁) and the β^{thal} . The phenotype of I₁ also seems to be anomalous: an inherited form of persistence of HbF coexists with a normal HbA/HbS ratio (see Table 1), although in every form of this condition so far described, there has always been total or partial differential depression of the β gene in the *cis* form, with respect to the HPFH mutation¹.

This is the first clear example in which a gene causing persistence of HbF does not behave as an allele of the $\delta\beta$ complex, as indicated by the fact that both β^s and HPFH sites, together in the gamete transmitted from I₁ to II₁, were present in the gamete transmitted from II₁ to III₁. Furthermore, only the HPFH site was transmitted from II₁ to III₂; in this case the locus for the persistence of HbF and that of β^{thal} previously separated, must have travelled together in the gamete which II₁ has transmitted to III₂. This individual carries both the genes for β^{thal} and for HPFH, as indicated by the high levels of HbA₂ and HbF. The latter finding cannot be explained by the variability of HbF in β^{thal} heterozygotes, because it has been shown that HbF levels show intrafamilial segregation in β^{thal} (ref. 9); and the grandfather of III₂, who transmits the high-A₂ β^{thal} gene, only shows 0.6% HbF. Moreover, the gene causing persistence of HbF does not alter the HbA/HbS ratio. Each of these two findings suggests that the site of mutation of the persistence of HbF in this family is located in a gene different from the classical HPFH gene.

The haematological, biochemical and genetic data of this family can be explained by the following assumptions: (1) The abnormality results from persistent γ -chain synthesis from one of the γ -chain loci in I₁ which passes on to III₁ through II₁. (2) The coexistence of both genes for β^{thal} and HPFH in III₂.

Table 1 Haematological data of family AMR

	Age (yr)	Hb (g%)	RBC (10^6 mm ⁻³)	Het (%)	MCV (μ m ³)	MCH (pg)	MCHC (%)	HbF (%)	HbA ₂ (%)	HbS (%)	Osmotic resistance	Serum iron (μ g%)	Intracellular distribution of HbF
I ₁	48	11.8	4.55	40	88	26	29.5	5.3	2.8	42	+	94	Heterogeneous
I ₂	53	12.8	6.16	47	76	21	27	0.6	5.0	—	++	106	—
II ₁	28	8.6	4.08	32	78	21	27	16.0	4.0	80	+++	118	Heterogeneous
II ₂	30	14.4	4.50	41	91	32	35	0.2	2.4	—	Normal	88	—
III ₁	9	13.8	5.15	44	85	27	31	5.2	2.9	42	+	103	Heterogeneous
III ₂	6	10.1	5.21	37	71	19	27	6.8	5.3	—	+++	100	Heterogeneous

+, Slightly increased; ++, increased; +++, very increased.

Haematological indexes and osmotic fragility were obtained by standard methods^{2,3}. Red blood cells (RBC) counts were performed in an Automatic Cell Counter (TOA Microcell Counter, Mod. CC-1002). Haemoglobin electrophoresis was performed according to the method of Smithies⁴. An electrophoretic technique was also used for the quantitation of HbA and HbS⁵. HbA₂ was quantitated by the chromatography method of Bernini⁶, and HbF by the alkali denaturation method of Betke⁷. The amount of serum iron was determined by the sulphonated bathophenanthroline method⁸.

is the consequence of a cross-over between the γ and β loci, a highly improbable event because of the linkage between the two loci^{10,11}. In fact, this would be the first example of a recombination between HPFH and β^s sites. (3) This hypothetical mutation, producing persistence of HbF, does not affect the expression of the β -structural gene in the *cis* form.

It is evident that many assumptions have to be made to fit all the data within the classical scheme. On the other hand, all the findings are to be expected assuming that the mutation under discussion is located in a locus which does not affect γ -chain expression by an intrachromosomal interaction. It is tempting to suggest that there exists a locus which affects the γ -chain expression *via* a diffusible substance. This would be the first example in mammals of a regulatory mechanism affecting the synthesis of a polypeptide chain by a diffusible substance.

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Sulphydryl groups as a new molecular probe at the $\alpha_1\beta_1$ interface in haemoglobin using Fourier transform infrared spectroscopy

THE haemoglobin molecule consists of a pair of subunits, each of which is made up of two different haem-bearing polypeptide chains, α and β . The contacts between the two $\alpha\beta$ subunit pairs are not covalent and constitute the $\alpha_1\beta_2$ (and $\alpha_2\beta_1$) interface. Symmetrical dissociation¹ of the haemoglobin molecule occurs relatively easily at this interface. Large changes in the $\alpha_1\beta_2$ contacts occur² as the molecule switches from the T to the R conformation during ligand binding³.

The association of the $\alpha_1\beta_1$ (and $\alpha_2\beta_2$) contact is much stronger. Although this contact is formed largely by nonpolar van der Waals interactions, asymmetrical dissociation along the $\alpha_1\beta_1$ interface is more difficult to achieve and little change occurs here during ligand binding². Reactions of the sulphydryl (SH) groups of cysteinyl residues α -104 (G11) and especially β -112 (G14), located at the $\alpha_1\beta_1$ interface, are very useful in dissociating the $\alpha\beta$ subunit^{4,5}.

These residues may also be involved in intracellular haemoglobin denaturation occurring in thalassaemia and unstable haemoglobinopathies as well as in interaction of denatured haemoglobin with cell membranes⁶. In spite of its importance to haemoglobin structure and stability, the $\alpha_1\beta_1$ interface and its hidden SH groups have remained inaccessible to available probes.

We report here the first observation of infrared absorption bands due to SH groups in a native protein. The observed infra-

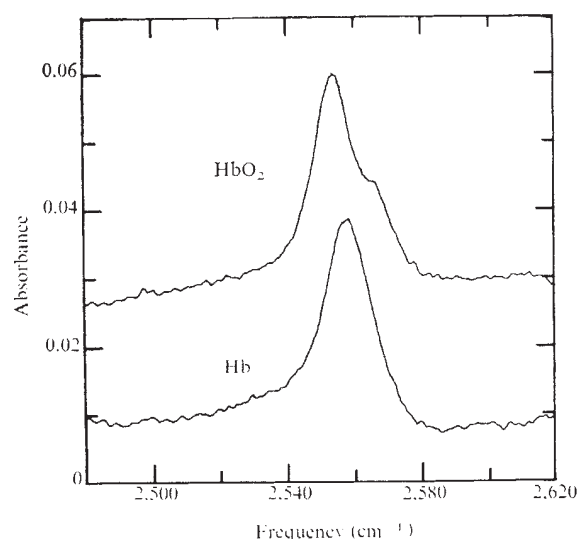


Fig. 1 Infrared spectra of 17 mM (haem) human oxyhaemoglobin and deoxyhaemoglobin against 16 mM bovine carboxyhaemoglobin and deoxyhaemoglobin, respectively. Single beam spectra of human haemoglobin samples were taken in a cell with a 0.2 mm optical path and CaF₂ windows using a Digilab Model FTS-14D interferometer equipped with a liquid nitrogen-cooled InSb detector. Four sets of 64 coherently added interferograms were independently subjected to Fourier transformation and were, in turn, co-added to yield a digital single beam spectrum at 2 cm⁻¹ resolution. This was initially compared with a single spectrum of air to produce an absorbance spectrum of the sample. A similarly obtained digital absorbance spectrum of H₂O was then subtracted from the sample spectrum. The water content of the samples was determined from a near infrared absorption band of H₂O at 1.92 μ m. Bovine haemoglobin spectra were treated similarly and subtracted from the human haemoglobin spectra. Haemoglobin solutions were buffered to pH 7.1 with 0.05 M bisTris and were 0.1 M in chloride.

red absorption bands are the result of S-H stretching vibrations of cysteine residues at the $\alpha_1\beta_1$ interface (α -104 (G-11) and β -112 (G-14)) of native human haemoglobin A. Centre frequencies and absorption intensities of these SH groups provide structural information about haemoglobin in aqueous solution that has been available in no other way.

The absorption bands due to cysteine SH groups at the α -104 (G-11) and β -112 (G-14) positions of human oxyhaemoglobin and deoxyhaemoglobin, respectively, are shown in Fig. 1. These were measured with Fourier transform infrared (FTIR) interferometry (with a Digilab Model FTS-14 Fourier transform spectrometer), and are corrected for absorptions due to water and to protein by subtraction of the appropriate spectrum of bovine haemoglobin, which contains cysteine only at the β -93 position⁷. The latter residue is predicted to have only a very weak, broad absorption band that is not observed in our conditions. The use of bovine haemoglobin as reference assures that only cysteine SH groups at the α -104 and β -112 positions in human haemoglobin will be observed. Assignment (J.O.A., G.H.B., and P.A.B., unpublished) of these absorption bands to S-H stretching vibrations is based on: first, comparison with small molecule thiols; second, loss of the absorption band at alkaline pH; and third, observation of a pair of bands with a similar absorption envelope near 1,855 cm⁻¹ in D₂O but not in H₂O, that have a calculated isotopic frequency ratio (ν^*/ν) equal to 0.7268, which is virtually identical to the corresponding values calculated from the data⁸ reported for methanethiol vapour (0.7267) and liquid (0.7270).

The spectrum of human oxyhaemoglobin SH groups (Fig. 1) clearly contains two overlapping bands of differing intensities. Quantitative band shape analysis shows that the apparently more symmetrical deoxyhaemoglobin SH spectrum is also composed of two overlapping bands. Horse and pig haemoglobins contain cysteine residues at only the α -104 and β -93 positions⁷. Their oxyhaemoglobin spectra, when compared with

Table 1 Infrared intensities of sulphhydryl absorptions

	ϵ_{mM} (area)* ($\text{mM}^{-1} \text{cm}^{-2}$)
0.1 M ethanethiol in CCl_4	0.070
0.1 M ethanethiol in H_2O	0.210
5.36 M <i>n</i> -propanethiol in acetone	0.62†
HbA: 8.5 mM $\alpha_1\beta_1$ dimer	
α -104 Cys (G-11)	2.7
β -112 Cys (G-14)	0.80

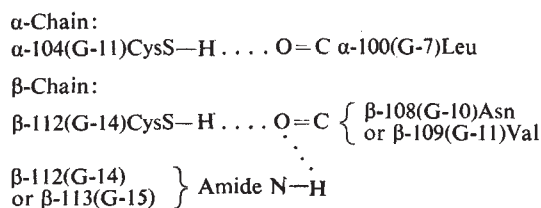
* ϵ_{mM} (area) = $[1/(\text{cl})] \int \log_{10} (I_0/I) dv$

† From ref. 10.

bovine haemoglobin (β -93 cys), show only a single symmetrical absorption band of similar frequency and intensity to the lower frequency, more intense, SH absorption band of human haemoglobin. A band corresponding to the lower intensity SH shoulder from human oxyhaemoglobin is not present in spectra from horse or pig. We therefore assign the band at $2,553.8 \text{ cm}^{-1}$ to $\nu(\text{SH})$ of the α -104 cysteine in human oxyhaemoglobin, and the shoulder due to a band at 2567 cm^{-1} to $\nu(\text{SH})$ of the β -112 cysteine. Corresponding bands are observed at $2,557.0 \text{ cm}^{-1}$ and $2,564 \text{ cm}^{-1}$ in human deoxyhaemoglobin. These frequencies are sensitive to the state of ligation of haemoglobin, and differ in the order $\text{HbCO} < \text{HbO}_2 < \text{HbCN} < \text{Hb}^+ \ll \text{Hb}$, for the α -104 $\nu(\text{SH})$ (J.O.A., G.H.B., and P.A.B., unpublished).

The reasons for the sensitivity of these sulphhydryl groups to small changes in molecular structure may be understood by consideration of the observed absorption intensities (Table 1). Monomeric thiols absorb very weakly in the infrared unless they are polarised by molecular interactions such as H bonding⁹. Thus, H bonding by solvent water produces about three times the integrated intensity observed in CCl_4 , and acetone produces a further threefold increase in intensity. The integrated intensity of propanethiol in acetone¹⁰ is somewhat less than that due to the β -112 cysteine SH, but less than one fourth of that due to the α -104 cysteine SH. We therefore propose that both of the SH groups at the $\alpha_1\beta_1$ interface in haemoglobin must be strongly H bonded, probably to a peptide carbonyl of the fourth residue back in the G helix. Such an H bond seems consistent with Perutz' suggestion that certain serine residues may form similar H bonds¹¹. This possibility was tested by construction of molecular models of the G helices for horse oxyhaemoglobin (Labquip Co., Caversham, UK) from coordinates attributed to

Perutz. The G helix of horse β chain contains no cysteine, and the amide NH groups all seem to be fairly well aligned with corresponding amide carbonyl groups four residues back. The same observation is made for the G helix of the horse α chain except for the amide carbonyl of α -100 (G-7 leu), which seems to stick out sharply from the helix (Fig. 2). This carbonyl is in a very favourable position to form a strong H bond with the SH group of the α -104 (G-11) cysteine, which would satisfy the infrared intensity requirements. We predict that a somewhat lesser distortion of the human β chain G helix may be found, which would permit an amide carbonyl at β -108 or β -109 to form a somewhat weaker H bond with the β -112 cysteine SH, which may be shared with an amide NH group of β -112 or β -113, respectively. A weaker H bonding of the β -112 cysteine SH group would agree with the weaker infrared absorption intensity and higher frequency of the band assigned to this group (Fig. 3). Polarisation and rotational fixation of the β -112 SH by H bonding may partially account for the greater reactivity with iodoacetate of the β -112 as compared to the β -93 sulphhydryl in isolated human haemoglobin β chains observed by Neer¹².

**Fig. 3** Proposed H bonding for the α -104 (G-11) and β -112 (G-14) cysteine SH groups in human haemoglobin.

This model can account qualitatively for the observed sensitivity of $\nu(\text{SH})$ to differences in the state of haem ligation. Change in quaternary structure upon ligation results in a relative displacement of atoms at the $\alpha_1\beta_1$ contact by about $1 \text{ \AA}$ (ref. 2). The contribution of altered van der Waals contacts to $\nu(\text{SH})$, however, should be small unless there is an alteration of polar group association with the sulphhydryls. Alternatively, changes in tertiary structure may be responsible. Slight bending of the G helix would be geometrically amplified to change the S-H $\dots$ O=C hydrogen bond distance (Fig. 2, arrow), or a slight rotation of the α -100 peptide carbonyl could cause a change in the strength of the hydrogen bond association. Although these possibilities remain to be tested, Fourier transform infrared spectroscopy of haemoglobin sulphhydryl groups clearly provides a sensitive molecular probe for the observation of structural changes in the $\alpha_1\beta_1$ interface region resulting from tertiary or quaternary conformational changes of the protein.

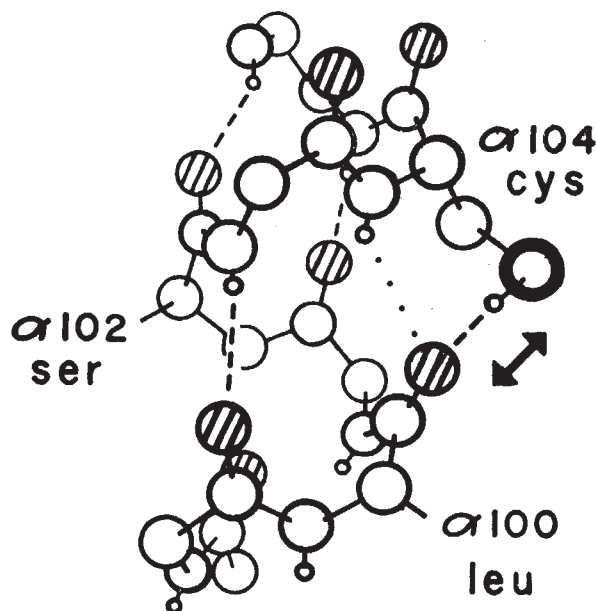
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**Fig. 2** Drawing of the α chain G helix of horse oxyhaemoglobin from α -98 (G-5) to α -105 (G-12), prepared from a photograph of a molecular model. The amino acid side chains are not included except for that of α -104 (G-11) cysteine. The oxygen atoms are shaded and the sulphur atom is in bold face.

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NMR evidence for tertiary structure base pair in *E. coli* tRNA involving S⁴U₈

In previous nuclear magnetic resonance (NMR) studies of tRNA we observed anomalously low field resonances (14.5–15.0 p.p.m. downfield from the usual standard 2,2-dimethylsilapentane-5-sulphonate (DSS)) in the spectra of many, but not all, *Escherichia coli* tRNA^{1–3} but yeast tRNA we examined never exhibited such resonances^{1,2}. The fact that approximately 60% of the *E. coli* tRNA are class I and have a 4-thiouridine (S⁴U) residue at position 8 (refs 4 and 5), whereas yeast tRNA do not, raised the possibility that the anomalous low field resonances in *E. coli* tRNA might be due to ring NH protons of S⁴U₈ in a tertiary structure base pair.

To test this possibility, NMR spectra of S⁴U and U mononucleosides were compared in dimethylsulphoxide (DMSO) and we found that the ring NH proton of U was at 11.5 p.p.m. (ref. 6) whereas the corresponding resonance of S⁴U was at 12.6 p.p.m. (K.L.W. and D.R.K., unpublished). Assuming comparable hydrogen bond strengths, these model system studies suggested that the ring NH proton of an S⁴U–A base pair would be located further downfield than the corresponding resonance of an AU base pair. As resonances from AU base pairs can be located as far downfield as 14.5 p.p.m. (refs 1–3), these observations prompted us to re-examine the assignment of the anomalous low field resonances in *E. coli* tRNA. For this study we chose to work with unfractionated tRNA to determine whether or not base pairing with S⁴U₈ is a common feature in *E. coli* tRNA. As we shall show, a tertiary structure base pair involving S⁴U₈ is responsible for the anomalous low field resonances in the NMR spectra of *E. coli* tRNA.

Unfractionated *E. coli* tRNA was obtained from Plenum Scientific (Lot no. 73042). Three different procedures were used to convert S⁴U to U, including IO₃[−] oxidation (K.L.W. and D.R.K., unpublished), photo-oxidation^{7,8} and cyanogen bromide treatment^{9,10}. The conversion of the S⁴U to U was monitored by following the disappearance of the S⁴U absorption spectrum and in each case at least 90% of the S⁴U residues were converted. NMR samples were prepared free of chemical reagents and then dialysed against solutions containing appropriate amounts of Mg²⁺, cacodylate and NaCl. A typical sample contained ~25 mg ml^{−1} of tRNA, 0.1 M NaCl, 10 mM Mg and 10 mM cacodylate buffer, pH 7. NMR spectra were obtained with a Varian HR 300 spectrometer operated in the field sweep mode. All spectra were extensively (1–2 h) signal averaged using a Nicolet 1020A signal averager. The low field NMR spectrum of the untreated *E. coli* tRNA is shown in Fig. 1 along with the spectra of the chemically modified samples. All spectra were taken at 25° C.

As expected on the basis of our earlier examination of several pure *E. coli* tRNA species, unfractionated *E. coli* tRNA exhibits a broad, rather weak resonance located around 14.8 p.p.m. with only ~0.025 times the intensity of the resonances located between 11.5 and 14.5 p.p.m. Resonances in the 14.5–11.5 p.p.m. region have previously been assigned to the ring NH protons of AU or GC Watson–Crick base pairs (one resonance per base pair)^{1–3}

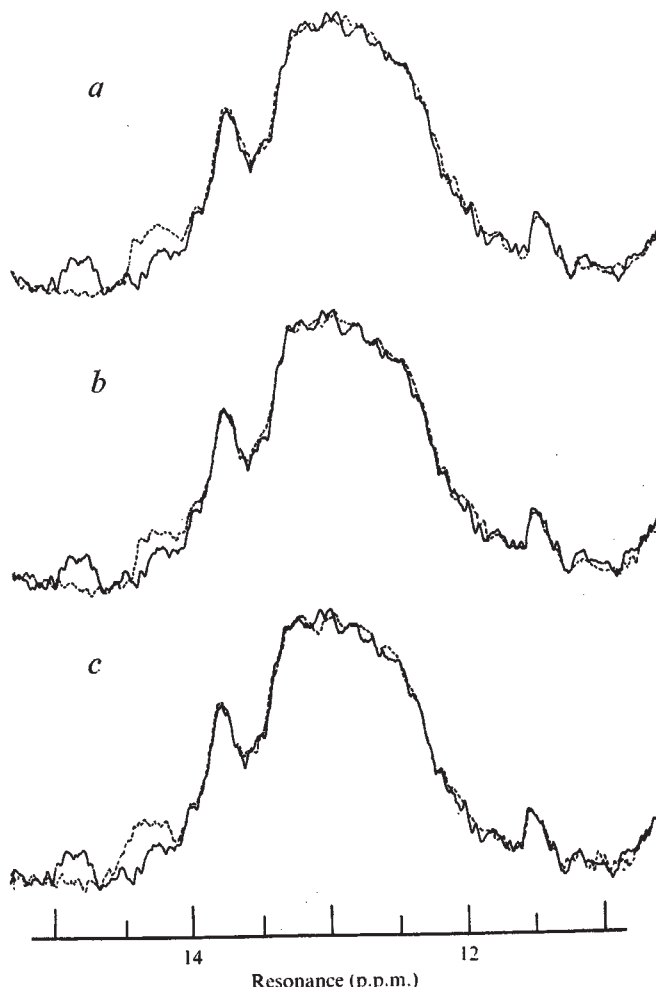


Fig. 1 A comparison of the 300 MHz proton NMR spectra of unfractionated *E. coli* tRNA before (—) and after (---) chemical conversion of S⁴U to U. Spectra were obtained at 25° C and the positions of the resonances are in p.p.m. downfield from DSS. Additional experimental details are given in the text. Three differential chemical procedures were used, including *a*, photo-oxidation; *b*, cyanogen bromide treatment; and *c*, IO₃[−] oxidation. As these comparisons demonstrate, the only major changes in the spectra occur in the 14.8 and 14.3 p.p.m. regions of the spectra. All other features in the spectrum remain virtually unchanged.

and integration of these spectra indicates there are an average of 20±2 resonances per tRNA^{1–3}. Thus, the intensity in the 14.8 p.p.m. region corresponds approximately to ~0.5 proton (base pair) per molecule. This number is consistent with our attribution of the 14.8 p.p.m. resonance to S⁴U as about 60% of the *E. coli* tRNA are class I and have an S⁴U residue at position 8 (refs 4 and 5).

To prove that S⁴U residues are responsible for the anomalous low field resonances three different chemical procedures were used to convert S⁴U to U. The effect of each of these on the low field NMR spectrum is shown in Fig. 1. In each case the S⁴U→U conversion causes the loss of the 14.8 p.p.m. resonance and the appearance of a new resonance at around 14.3 p.p.m. On the basis of these observations we conclude that the anomalous 14.8 p.p.m. resonance in *E. coli* tRNA is due to a tertiary structure base pair in which S⁴U₈ is hydrogen bonded with another base located elsewhere in the molecule. Consideration of the low field position of the resonance (before and after conversion to U), and the relative strengths of in-plane ring current shifts from G and A suggest that an adenine is the other base paired with S⁴U₈.

The following observations suggest that A at position 14 is the one paired with S⁴U₈. It has been demonstrated that

S⁴U₈ can be photochemically crosslinked with C₁₃ in several different *E. coli* species without significantly affecting the biological activity^{11,12}. As expected, we also observed that photocrosslinking S⁴U₈ and C₁₃ resulted in loss of the 14.8 p.p.m. resonance (K.L.W., and D.R.K., unpublished). Furthermore, conversion of S⁴U to U has little effect on the biological activity of the *E. coli* tRNA (refs 8 and 10). Taken together, these results indicate that in a biochemically active conformation the arms of the cloverleaf are folded with S⁴U₈ located close to C₁₃ and therefore also close to the A residue which is always present at position 14 (refs 4 and 5). On this basis, the 14.8 p.p.m. resonance is assigned to a tertiary structure base pair between S⁴U₈ and A₁₄. The formation of such a base pair was proposed some time ago by Levitt³ on essentially the same grounds, but this is the first experimental evidence that S⁴U₈ is actually involved in a tertiary structure base pair. Recent X-ray diffraction data show that A₁₄ and U₈ are close together in yeast tRNA^{Phe} crystals, but the resolution is not adequate to show whether they are actually hydrogen bonded^{13,14}. As a new resonance appears in the *E. coli* tRNA spectrum at 14.3 p.p.m. when S⁴U₈ is converted to U₈, there should be a similar resonance from a tertiary structure A–U₈ base pair in yeast tRNA. Re-examination of previously published spectra indicates that this is probably true¹⁻³.

Based on Leonard's structural studies of the S⁴U–C photodimer^{15,16} and his model for the arrangement of S⁴U₈ with respect to C₁₃ in tRNA (ref. 17), and previous NMR studies of yeast tRNA^{Phe} which indicates that A₁₄ is stacked on GC₁₃ (ref. 18) we have to conclude that the S⁴U₈–A₁₄ base pair is not Watson–Crick. If a few additional tertiary structure base pairs can be identified, then it may be possible to determine the complete folding of tRNA molecules in solution, particularly when this information is combined with the results of rare earth metal binding studies^{19,20}. Because the A–S⁴U(U) tertiary structure base pair is not part of a regular double helix it should be very sensitive to solvent conditions (salt, Mg²⁺, temperature) which are known to stabilise or destabilise the folding of tRNA. A detailed re-examination of the NMR spectra of some pure species and further work on chemical modifications is currently under investigation.

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Possible role of DNA synthesis in formation of sister chromatid exchanges

As there is now evidence that an eukaryote chromosome is composed of a single molecule of double-stranded DNA^{1,2}, sister chromatid exchanges (SCEs) detectable in mitotic chromosomes of higher organisms can be regarded as a kind of recombination between two homologous DNA duplexes. The SCE formation is readily enhanced by exogenous agents such as ultraviolet light and chemical mutagens and seems to be closely related to post-replicative repair process in mammalian cells³. Precise knowledge about the time and sites of the SCE formation might help greatly to understand the mechanism underlying this phenomenon and its biological significance. Previous attempts³ to pinpoint cellular phases in which this recombination process is initiated have been hampered by the usage of ³H-thymidine which, although indispensable for the demarcation of sister chromatids, would cause strand scissions at any cellular phases.

A chromatid consisting of DNA substituted with 5-bromodeoxyuridine (BUdR) can be clearly distinguished from one containing no BUdR using fluorescence staining techniques³⁻⁵, and visible light causes strand breaks in BUdR-substituted DNA regions^{6,7}. Taking advantage of these facts I here present circumstantial evidence that the site of the SCE formation is strictly limited to DNA portions which are undergoing or just have completed replication.

Chinese hamster cells growing exponentially, D-6³, were labelled with BUdR at various concentrations for 5 h in the dark, washed and incubated further for 21 h in fresh medium containing 10 µg ml⁻¹ thymidine, and then fixed for chromosome preparation following 1 h pretreatment of cells with 0.05 µg ml⁻¹ colcemid. The slides were stained with 0.125 mg ml⁻¹ acridine orange⁸. Most metaphase cells were in the second post-labelling mitosis (generation time; 16 h). Visible light illumination to cells labelled with BUdR was carried out at room temperature during the second post-labelling cell cycle at various time intervals from collection. The light source was a 20 W blue fluorescent light bulb (National) which emitted no ultraviolet light. Culture dishes containing 2 mm deep medium were placed at a distance of 5 cm from the bulb. The energy flux of the incident light in these conditions has not been determined. DNA synthetic activity of cells was determined autoradiographically in BUdR-substituted duplicate cultures by labelling cells with ³H-thymidine during the period of visible illumination.

Effects of the concentration of BUdR and visible illumination on the frequency of SCEs are shown in Fig. 1 and Table 1. In non-illuminated controls, the frequency of SCEs remained unchanged at BUdR concentrations 0.25–2.5 µg ml⁻¹. At higher concentrations it increased with increasing concentration. Visible illumination readily provoked SCEs in cells labelled with 1.0 µg ml⁻¹ BUdR. In the following experiments,



Fig. 1 Chinese hamster chromosomes stained with acridine orange. The cell was labelled with BUdR ($1.0 \mu\text{g ml}^{-1}$) for 5 h and illuminated with visible light in the second post-labelling cell cycle for 20 min at 8 h before collection ($\times 1,680$).

cells were labelled exclusively with $1.0 \mu\text{g ml}^{-1}$ BUdR and received 20 min visible illumination.

Figure 2 shows the time relationship of DNA synthetic activity of cells and the incidence of SCEs in No. 1 chromosomes. It is clear that the formation of SCEs was enhanced only when visible illumination was carried out in the S phase. As for the failure to induce SCEs in G₂, there may be a possibility that the G₂ chromatin might be resistant to the photolysis by visible light because of its condensed state and protection by chromosomal proteins. I thought this unlikely, however, as chromosomal aberrations were readily induced in cells illuminated with visible light at G₂. Without illumination, the frequency of chromatid breaks and achromatic lesions was about 0.03 per cell whereas in cells illuminated at 2 h before collection, it was 0.28 per cell.

The parallel between the increase in SCE frequency and in DNA synthetic activity was also found in the X chromosome (Fig. 3a). The X of Chinese hamster is a biarmed chromosome, the short arm replicates in the earlier part of S while the long arm in the later part of S. The X as a whole starts DNA synthesis about 2 h behind the other chromosome complements such as No. 1 chromosomes (Figs 2 and 3a). The increase in the SCE frequency over the control level was detected only when this particular chromosome was under replication. SCEs induced by visible illumination were observed exclusively

in the short arm in the earlier part of S whereas in the long arm in the later part of S. Figure 3b shows the ratio of the long arm to the whole X with respects to SCE frequency and DNA synthetic activity. The time when the curves for SCE ratio (Fig. 3b, A and B) crosses the control line is not appreciably different from the time when the ratio for DNA synthetic activity cross the average line; that is, when the majority of DNA-synthesising sites moved from the short arm to the long arm. This seems to indicate that strand breaks responsible for the initiation of recombination process might be those induced in DNA regions which are replicating or have just completed replication. The majority of breaks caused by visible light in BUdR-substituted strands may be repaired without strand exchanges between chromatids⁷. It is uncertain whether the concurrence of the SCE formation with DNA replication is due to a direct involvement of replication forks in the recombination process or due merely to geometrical constraint in terms of the possibility of the interaction of two broken ends; separation of duplicated DNA strands and their subsequent binding with chromosomal proteins might reduce greatly such a probability.

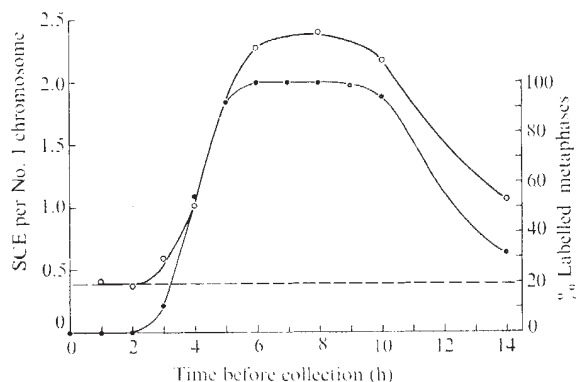


Fig. 2 The formation of SCEs in No. 1 chromosomes in relation to DNA synthetic activity of cells. ●, DNA synthetic activity is expressed as the % labelled metaphases. BUdR-substituted cells were labelled with ^3H -thymidine ($0.6 \mu\text{Ci ml}^{-1}$, specific activity 12.1 Ci mol^{-1}) for 20 min during the second cell cycle while they were being illuminated with visible light. ○, The SCE frequency was scored in cultures untreated with ^3H -thymidine. — —, Non-illuminated control.

The labelling procedure used here was such that only one of four strands in a metaphase chromosome contained BUdR. As the target of the photolysis by visible light is BUdR-substituted DNA portions, the results obtained in this work may seem to imply that a single break in a BUdR-substituted parental strand is sufficient to initiate a process of the SCE formation. The following alternatives cannot be precluded, however. First, a small amount of BUdR, which is undetectable by the present technique, would be incorporated into other three strands probably at the time of repair of spontaneous DNA lesions⁸, thereby providing two breaks upon visible illumination to initiate the recombination process. Second, visible illumination might cause strand breaks also in DNA unsubstituted with BUdR, though far less efficiently than in BUdR-substituted DNA⁶, and the SCE formation is initiated by two breaks each made in sister chromatids. As to the latter possibility, it was confirmed that at least DNA lesions detectable as chromosomal aberrations were not caused by visible illumination in cells untreated with BUdR: The number of breaks in cells illuminated with visible light 6 h before collection was found to be 0.06 per cell. This value was not significantly different from that obtained in non-illuminated controls (0.02 chromatid breaks per cell).

Table 1 Frequencies of SCEs in BUdR-labelled cells with or without visible illumination during the second post-labelling cell cycle

BUdR ($\mu\text{g ml}^{-1}$)	Visible illumination* (min)	SCEs per No. 1 chromosome†
0.25	—	0.44
0.5	—	0.45
1.0	—	0.44
2.5	—	0.42
5.0	—	0.58
10.0	—	0.85
20.0	—	1.62
40.0	—	2.61
1.0	0	0.44
1.0	5	1.58
1.0	10	2.12
1.0	20	3.22

* Illumination was carried out 8 h before fixation of cultures.

† 100 chromosomes each were scored in non-illuminated controls and 50 chromosomes each in visible illuminated cells.

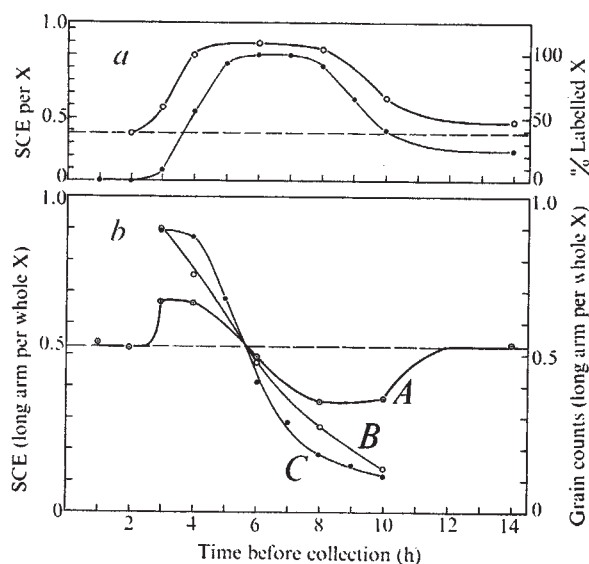


Fig. 3 The formation of SCEs and DNA synthetic activity in the X chromosome examined in the same samples as those used in Fig. 2a, time relationship of the enhancement of the SCE formation by visible light (○) and DNA synthetic activity (●) of the X chromosome. *b*, Ratios for the SCE frequency and DNA synthetic activity in the long arm of the X to those in the whole X. The SCE ratio in non-illuminated control was 0.524, whereas the average ratio for DNA synthetic activity was 0.526. The latter value was obtained by dividing the sum of the number of silver grains scored on the long arm in each sample by that on the whole X. Both ratios are indicated as a single broken line. Curve A shows the SCE ratio determined based on the net frequency of SCEs and curve B that based on the fraction of SCEs solely due to visible light illumination. Curve C indicates the ratio for DNA synthetic activity assessed by grain count.

The simplest and most attractive models for recombination presume two strand breaks as an initial step which is then followed by a heteroduplex formation by two broken ends thus derived⁹⁻¹¹. A speculation that a discontinuously replicating DNA piece at the replication fork might provide a pairing mate to a broken end in a BUDR-substituted strand seems to be attractive and awaits more detailed studies in this respect.

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Localisation of 5S ribosomal RNA genes on human chromosome 1

We have used *in situ* hybridisation to locate a major 5S RNA locus near the end of the long arm of chromosome 1 of man. This information represents a step towards the understanding of the synthesis, assembly and regulation of ribosomes. The

5S RNA molecule is associated with the larger ribosomal particle in both higher and lower organisms and is required for ribosome function. Ribosomal RNA—18S and 28S—is synthesised at the nucleolar organiser regions on the chromosomes of the D and G groups¹. Genes coding for 5S RNA have been located in *Drosophila*², *Xenopus*³ and several other organisms⁴, and our data now confirm preliminary reports^{5,6} that placed the 5S genes on chromosome 1 of man. We also suggest a mechanism for positioning the 5S genes near nucleoli at interphase.

Hybridisation experiments with human DNA on nitrocellulose filters have shown that DNA from the largest chromosomes has more 5S RNA genes than that from chromosomes in the other three size classes⁷. We have used the recently reported method for localising 5S genes more precisely by RNA:DNA annealing to metaphase figures, using high specific activity 5S RNA labelled with ¹²⁵I. The 5S RNA was isolated from polysomes of HeLa cells and purified by preparative gel electrophoresis. After iodination⁸ the RNA was repurified on another column (DEAE cellulose), dialysed extensively against water and lyophilised. This 5S RNA (specific activity about 2×10^8 d.p.m. μg^{-1}) was reconstituted in $4 \times \text{SSC}$ (0.15 M NaCl, 0.015 M sodium citrate) at pH 6.0 at a concentration of $2 \mu\text{g ml}^{-1}$. The nucleotide sequence of this HeLa 5S RNA has been determined⁹ and it was at least 90% pure 5S RNA.

Slides were prepared from cultured leukocytes taken from the peripheral blood of two individuals. We used standard methods involving methanol-acetic acid fixation. Just before hybridisation the chromosomal DNA on slides was denatured in 0.2 N HCl for 20 min. at room temperature, chilled immediately in $0^\circ\text{C } 2 \times \text{SSC}$, dehydrated in ethanol and air dried. The reconstituted 5S RNA solution was diluted with an equal volume of formamide and used for annealing experiments with chromosome preparations. Background radioactivity and nonspecific binding are reduced considerably if 1-4 mg of commercial *Escherichia coli* tRNA or yeast 4S and 5S RNA is added to 1 ml of hybridisation mixture. Other details of the procedure have been described⁸. Autoradiographs were prepared using a 1:1 (w/w) dilution of Kodak NTB with distilled water, exposed for 2 months, developed by standard methods and stained with Giemsa in Sorenson's buffer at pH 7.0.



Fig. 1 Autoradiographs of human chromosomes of the A group hybridised *in situ* with ¹²⁵I-5S RNA. The horizontal lines indicate sets of A group chromosomes from six different cells. The diagrammatic chromosomes agreed on by the Paris conference¹⁰ are on the left. (The major chromosome section numbers are indicated but the sub-numbers for individual bands have been deleted because of the low magnification here.) The 5S genes are placed near q41 or q42 on chromosome 1, which correspond to the first proximal band and interband in 1 q4 indicated by the arrow. The 'noise level' radioactivity on chromosome 2 is randomly distributed. A minor 5S gene site may be on chromosome 3 but the data are inconclusive.

In almost every cell examined, chromosome 1 was labelled at a site near the end of the long arm (q) with an average of 2.8 silver grains per locus. In Fig. 1 the chromosomes in the A group from six cells are compared with the standardised chromosomes agreed at the Paris chromosome conference¹⁰. Measurements of chromosome 1 that exhibit clear banding at 1q12 place the 5S genes on the long arm at about 1q41. The location of this 5S gene site has been confirmed using ¹²⁵I-5S RNA to label the chromosomes from two fibroblast cultures, which were heterozygous for different translocations¹¹. With one aberration, t(1;13) (q32;q34), the 5S locus on chromosome 1 was translocated to chromosome 13. In the second aberration, t(1;4) (q44;q25), the 5S genes were not translocated to chromosome 4. The data are all consistent in placing the major 5S RNA locus on the long arm of chromosome 1 at or near q41 and within the segment q32 to q44 as bracketed by translocation breakpoints.

The localisation of 5S genes on the remaining chromosomes of the genome is less straightforward. An identified chromosome must be labelled consistently at a particular site, otherwise the data are meaningless. It has not been possible to identify labelled chromosomes with certainty because of variable banding patterns. The information that follows is an attempt to provide tentative assignments for the 5S genes, and provides a qualitative analysis of each chromosome group (A–G) shown in Fig. 2, obtained from the karyotypes of 34 cells at metaphase (20 male and 14 female). (A) Chromosome 1 is the most heavily labelled. Chromosome 2 is not labelled above background, however, 3 may be labelled but at a frequency of silver grains too low to place a site. (B) Chromosomes 4 and 5 are not labelled above noise level binding. (C) One or two pairs of the middle sized chromosomes are labelled. In cells showing distinct C banding, chromosome 9 is usually labelled.

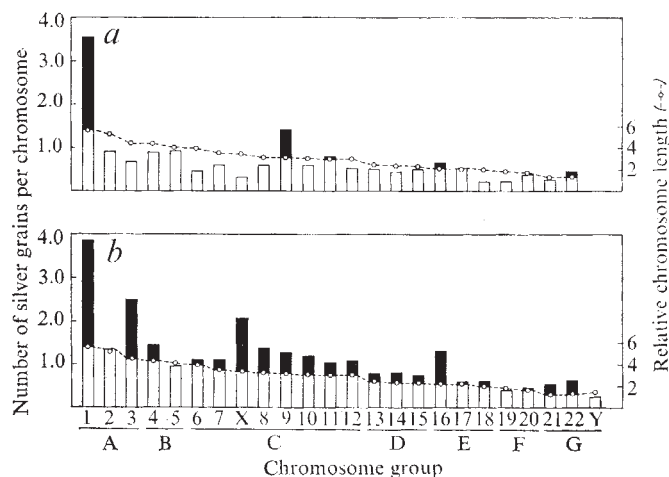


Fig. 2 An analysis of autoradiographs from metaphase chromosomes hybridised with ¹²⁵I-5S RNA. Radioactivity and relative chromosome length are plotted according to chromosome number. Chromosomes 1, 2, 3 and 16 are identified with reasonable certainty. The placement of the remaining chromosomes is reliable only within a group according to their measured length. This latter placement is analogous to the older criteria before G and C banding techniques were available. The assignment of the chromosomes in the top set (female 40°C) is more accurate because some chromosomes had prominent C bands, which apply especially to assignments within the C group. *a*, These chromosomes are from cultured leukocytes of an adult female. The hybridisation with 1 µg ¹²⁵I-5S RNA per ml was done at 40°C in 50% formamide in 2×SSC overnight. *b*, These chromosomes are from cultured leukocytes of an adult male. The hybridisation conditions are the same, except that the slides were incubated at 60°C for one hour before transfer to the optimum temperature at 40°C overnight. A 60°C temperature in 50% formamide will melt the 5S DNA. We believe that some non-histone proteins are removed with these conditions because of increased hybridisation efficiency.

When the X chromosome can be identified by banding, it is not labelled. (D) Chromosomes 13, 14 and 15 do not seem to carry 5S genes nor do detected levels of annealing occur at the satellited regions that would indicate contamination from 18S or 28S ribosomal RNA. (E) Chromosome 16 probably has 5S genes. The identification of 16 is reliable because the centromere is located centrally and there is a large C band at q11. Chromosome 17 and 18 are not labelled above background. (F) Neither 19 nor 20 are labelled. (G) Chromosome 22 might have 5S genes but 21 and the Y probably do not, and the Y exhibits less 5S RNA binding per unit length than any other chromosome. Thus besides the definite localisation on 1, tentative assignments indicate that chromosomes 9 and 16 probably have 5S genes. Chromosomes 3 and 22 have above average labelling but remain in question. The overall distribution of the 5S genes is consistent with previous hybridisation studies⁷.

In interphase nuclei the 5S RNA genes have been observed to cluster around nucleoli in *Drosophila*^{12,13}, *Xenopus*³ and the Chinese hamster¹¹. (We have verified these results in the Chinese hamster, where the 5S RNA genes are clustered around nucleoli.) With interphase nuclei from mouse Sertoli cells, human leukocytes and human fibroblasts the radioactivity from annealed 5S RNA is associated with nucleoli¹⁵. An analysis of interphase nuclei from 100 human fibroblasts shows that 50–70% of the ¹²⁵I-5S RNA radioactivity is associated with nucleoli. A random distribution would be 20–30%. Centres of radioactivity and lines of 6–10 silver grains are usually over nucleoli and at the edge¹¹. Many of these highly labelled sites should be the 5S genes on chromosome 1. Most nuclei with 3 or 4 nucleoli have label associated with each nucleolus, indicating that more 5S genes are present than on chromosome 1.

The obvious advantage of this nucleolar association would be to synthesise 5S RNA in proximity of nascent particles for effective assembly of 5S RNA into the 60S ribosomal particles. Interphase nuclei seem to possess a mechanism to position certain chromosome segments into a consistent three-dimensional configuration. What is the physical basis for the association of the ribosomal precursor genes?

Certain information about human chromosomes—(a) banding patterns¹⁰; (b) the location of C bands and the highly repeated DNA sequence¹⁶; (c) the localisation of the ribosomal RNA gene at the secondary constrictions of the D and G group chromosomes 13, 14, 15, 21 and 22¹, and (d) the 5S gene sites described here—shows that the chromosomes with large blocks of highly repeated DNA (C bands) are either nucleolus organiser chromosomes (13, 14, 15, 21 or 22) or chromosomes 1, 9 and 16, reported here to have 5S genes. C-RNA from satellite II anneals to the C band on chromosome 1 at q12 and with the C band on chromosome 16 at q11¹⁷. After long exposure, these same *in situ* preparations gave labelling at the C band on chromosome 9 at q12 and at the nucleolar heterochromatin. Are these highly repeated DNA sequences the recognition sites which position the ribosomal precursor genes?

Ferguson-Smith and Handmaker¹⁸ analysed the position of the nucleolus organiser (NO) chromosomes in relation to the location of other chromosomes in 432 mitotic cells. Chromosome 1 was consistently associated with the NO chromosomes, and direct attachments were illustrated between the C band q12 on chromosome 1 and the nucleolar heterochromatin end of a chromosome in the D or G group (Plate 3A–C of ref. 18). Shaw made a similar observation¹⁹ where the D and G group chromosomes were adjacent to chromosome 1, usually at right angles to the acentric ends of the D or G chromosomes that point toward the centromere of chromosome 1. The evidence suggests that the highly repeated DNA sequences function to orient and position the genes which synthesise the RNA for ribosomes.

Even though the assignments of 5S genes to chromosomes 9 and 16 will require further cytogenetic verification before

their mapping can be accepted, some observations on association should be noted. Using high pH Giemsa staining the large heterochromatic block on chromosome 9 could be recognised at interphase in fibroblasts and Sertoli cells²⁰. This block was often associated with the small nucleoli of fibroblasts and showed a frequency of 84% attachment of Sertoli cell nucleoli. In seven consecutive patients with chronic myelogenous leukaemia, Rowley²¹ found that a piece of chromosome 22, an NO chromosome, was translocated to chromosome 9. These two chromosomes must have been closely associated at interphase to produce the translocation.

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Pseudotype formation between enveloped RNA and DNA viruses

ENVELOPED RNA viruses from different groups participate readily with each other in phenotypic mixing during maturation at the plasma membrane of host cells. These 'pseudotypes' with the envelope antigens of one virus and the genome of the other have been demonstrated for simian virus 5 and vesicular

stomatitis virus (VSV)¹, for avian or murine RNA tumour viruses and VSV²⁻⁴, and for fowl plague virus and VSV⁵. (An exception has been the lack of interaction between Sindbis virus and VSV⁶.) We have now found virions containing the RNA genomes of VSV in the envelope antigens of a DNA virus, herpes simplex virus (HSV). These interactions between genomes and antigens of unrelated groups may be part of a mechanism for viral pathogenesis and oncogenesis.

Coinfection of cells with HSV and VSV would be expected to result in progeny containing either the HSV or VSV genomes. To differentiate between plaques, the morphology and efficiency of plaque formation by VSV and HSV were examined. HSV was assayed on both human epidermoid number 2 (HEp-2) cells and on Chinese hamster ovary (CHO) cells under liquid or agar overlays, and VSV was assayed on both cell types under agar only. The results of many experiments are summarised here.

The MP strain of HSV⁷ formed no detectable plaques on CHO cells. (We show below that this restriction was due to the inability of HSV to enter CHO cells.) The Indiana serotype of VSV⁸, on the other hand, grew well on both types of cell, although the efficiency of plaque formation on HEp-2 cells was only 10% of that on CHO cells.

The MP strain of HSV forms polykaryocytes on HEp-2 cells when the cells are incubated under liquid overlay containing anti-HSV antiserum⁷. Under agar, HSV on HEp-2 cells appeared as very small, red plaques and small, clear white plaques. HSV plaques were similar whether the agar overlay contained anti-HSV antiserum or not. The white plaques, although similar in size to VSV plaques on HEp-2 cells, were distinguishable by their appearance under the microscope.

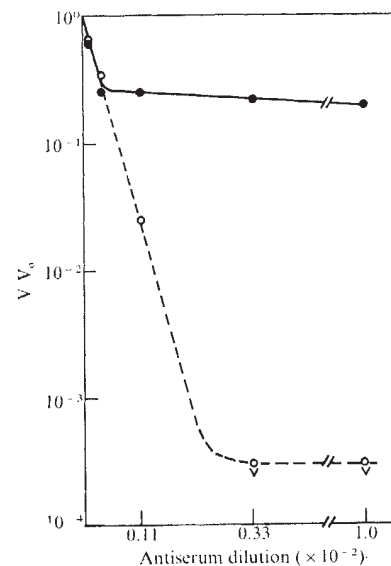


Fig. 1 Neutralisation by anti-VSV serum of VSV(HSV) pseudotypes formed after mixed infection in the presence of cytosine arabinoside. HEp-2 cells were infected with HSV at a multiplicity of 2 in the presence of 20 $\mu\text{g ml}^{-1}$ of cytosine arabinoside and incubated at 37° C for 4.5 h. Then the cells were superinfected with VSV at a multiplicity of 0.01. After each attachment period the cells were washed twice with 10 ml of saline. The cells were covered with MEM containing 2% FCS, 5 $\mu\text{g ml}^{-1}$ gentamicin and 20 $\mu\text{g ml}^{-1}$ cytosine arabinoside. After a total incubation time of 30 h at 37° C, the extracellular fluid was decanted, cells and cell debris precipitated and the supernatant frozen at -100° C. For neutralisation mixtures the supernatant containing VSV and VSV(HSV) progeny was thawed and mixed with equal volumes of rabbit anti-VSV antiserum³ at the indicated dilutions. Neutralisation was for 1 h at 22° C and then the samples were diluted and plaque assayed on either HEp-2 or CHO cells as indicated for Table 1. The log of the percentage of non-neutralised virus V/V₀ is plotted against the antibody concentration. ●, Hep-2; ○, CHO.

Table 1 Assay of VSV progeny produced by HEp-2 cells coinfectd with VSV and HSV or infected with VSV alone

Virus inoculum	Anti-VSV*	Anti-HSV†	Assay cells	PFU ml ⁻¹ × 10 ⁻³
VSV + HSV	—	—	HEp-2‡	3.40
VSV + HSV	+	—	HEp-2	1.10
VSV + HSV	—	+	HEp-2	1.80
VSV + HSV	+	+	HEp-2	<0.01
VSV	—	—	HEp-2	19.00
VSV	+	—	HEp-2	<0.01
VSV	—	+	HEp-2	17.00
VSV	+	+	HEp-2	<0.01
VSV + HSV	—	—	CHO§	2.25
VSV + HSV	+	—	CHO	<0.01
VSV	—	—	CHO	5.00
VSV	+	—	CHO	<0.01

A sample of 4.5×10^5 human epidermoid number 2 (HEp-2) cells¹⁷ were infected with the MP strain of herpes simplex virus type 1 (HSV-1)⁷ at a multiplicity of 1 and superinfected 4 h later with vesicular stomatitis virus (VSV) of the Indiana serotype at a multiplicity of 0.4. The infected cells were incubated in Eagle's minimal essential medium (MEM) containing 10% foetal calf serum (FCS) and 5 µg ml⁻¹ of gentamicin. After 24 h extracellular virus was collected as previously described¹⁸. A similar sample of HEp-2 cells was mock-infected with HSV then infected with VSV at a multiplicity of 0.4 and collected at the same time as the previous sample. Neutralisation mixtures were made by first diluting antiserum against VSV 1:50 and antiserum against HSV 1:100. Each serum was mixed in equal volumes with each of the two virus preparations which had been diluted previously to yield approximately 10^3 – 10^4 PFU ml⁻¹ on the respective cells. Where both antisera were used against one of the virus preparations, both concentrations of antisera were each identical to the concentration of antiserum when used alone. Neutralisation mixtures were allowed to stand at 22° C for 1 h and then diluted. Unneutralised virus preparations were treated identically except that antisera were absent. Virus in duplicate aliquots of 0.1 or 0.2 ml of the appropriate dilutions were attached to monolayer HEp-2 or Chinese hamster ovary (CHO) cells on 60 mm plastic Petri plates for 40 min at 37° C. The cells were then rinsed with medium and overlaid with MEM containing 2% FCS, 5 µg ml⁻¹ of gentamicin and 0.9% agar. After incubation at 37° C for 48 h in a 5% CO₂ humidified incubator, the cells were stained with 0.01% neutral red and the plaques counted after another 18 h of incubation.

* Rabbit anti-VSV antiserum was prepared as described previously³.

† Anti-HSV serum was human immune serum globulin provided by Dr Michael Oxman and prepared by the Department of Public Health of the Commonwealth of Massachusetts.

‡ HEp-2 cells and their properties have been described before¹⁹.

§ CHO cells and their properties have been described^{18, 20}.

The efficiency of plaque formation of HSV with a liquid or agar overlay was approximately the same.

When HEp-2 cells were infected with HSV and later superinfected with VSV, results were different depending on the multiplicity of VSV. At high multiplicities (>1) only progeny VSV were detectable in the extracellular medium. These progeny were neutralised by anti-VSV serum whether they were assayed on HEp-2 or CHO cells.

During mixed infection with smaller multiplicities of superinfecting VSV (<1) both VSV and HSV progeny were detected in the extracellular medium. Table 1 shows the results when VSV plaques were recorded. On HEp-2 cells one-third of the VSV progeny produced by the mixed infection was resistant to neutralisation by anti-VSV serum. This resistant VSV population did not form plaques on CHO cells and was neutralisable by anti-HSV serum when assayed in HEp-2 cells. A control sample of progeny from HEp-2 cells infected with only VSV is presented for comparison. Anti-HSV antiserum had no effect on this VSV preparation. These results demonstrate the presence of virions containing VSV genomes covered by HSV envelope antigens which are designated pseudotypes of VSV(HSV).

To ensure that there was no confusion between the VSV and HSV plaques in this experiment, we took advantage of reports that cytosine arabinoside inhibits HSV DNA synthesis without marked inhibition of the synthesis of plasma membrane-associated HSV glycoproteins^{9,10}. Because our experiments

were designed to assay only released extracellular virions, the growth of HSV for 28 h under these conditions was determined in the presence of 1–50 µg ml⁻¹ cytosine arabinoside. The growth of detectable extracellular HSV progeny was inhibited by more than 99.9% at these concentrations of cytosine arabinoside (data not shown). Less than 10° PFU per ml was detected. The same inhibitor at 20 µg ml⁻¹ had no effects on the synthesis of VSV in HEp-2 cells, nor did it result in marked cytotoxicity of uninfected HEp-2 cells after a 30 h treatment at 37° C.

HEp-2 cells coinfectd with HSV and VSV in the presence of cytosine arabinoside should synthesise HSV glycoproteins, but the progeny should contain only those with VSV genomes. To detect VSV(HSV) pseudotypes three different protocols were used for these mixed infections. In each case HEp-2 cells were infected with HSV at a multiplicity of 2 in the presence of 20 µg ml⁻¹ cytosine arabinoside. These cells were superinfected with VSV at a multiplicity of 0.01, 4.5 and 6.5 h after HSV infection. A third sample was superinfected, 22 h after HSV infection, with VSV at a multiplicity of 50. All three samples were collected 30 h after HSV infection, when the cytopathic effect of VSV was evident.

Of the infectious progeny from these mixed infections 3–20% were resistant to neutralisation by anti-VSV serum. A more detailed characterisation of the sample superinfected with VSV at 4.5 h is shown in Fig. 1 and Table 2. A persistent fraction of 20% was detected on HEp-2 cells after neutralisation with anti-VSV serum (Fig. 1). This persistent fraction was not detected on CHO cells (Fig. 1). Such findings indicate a surface restriction^{2,3} for VSV(HSV) on CHO cells and may explain the lack of susceptibility of some Chinese hamster cells to HSV^{11,12}.

A control preparation of VSV made in HEp-2 cells in the presence of 20 µg ml⁻¹ cytosine arabinoside was neutralised by anti-VSV serum (Table 2). In addition, VSV(HSV) from the mixed infection was neutralised specifically by anti-HSV serum (Table 2).

These results confirm the findings that specific alteration of host plasma membranes by HSV occurs although HSV progeny do not use plasma membranes during their maturation^{13–15}. Presumably, during budding VSV genomes pick up HSV-specific antigens. Also, VSV(HSV) pseudotypes indicate that enveloped DNA and RNA viruses mix phenotypically. The process appears to be quite efficient because 30% of the VSV population can be pseudotypes. It remains to be shown whether this percentage will be as high when strains other than the macroplaque (MP) strain of HSV are used in mixed infections.

If pseudotype formation occurs between VSV and other herpes-like viruses, the rescue of membrane markers by VSV from human tumour cells¹⁶ might be the result of partial gene

Table 2 Neutralisation of pseudotypes by human immune globulin

Virus	Anti-VSV	Anti-HSV	PFU ml ⁻¹ × 10 ⁻³
VSV-CA*	—	—	1.250
VSV-CA	+	—	<0.001
VSV(HSV)†	—	—	9.820
VSV(HSV)	+	—	0.475
VSV(HSV)	—	+	8.620
VSV(HSV)	+	+	<0.001

Neutralisation mixtures were made with equal volumes of virus and anti-VSV and/or anti-HSV sera. Details of neutralisation and plaque assay on HEp-2 cells were identical to those described for Table 1.

*VSV-CA was prepared in 5×10^6 HEp-2 cells by first treating the cells with cytosine arabinoside at 20 µg ml⁻¹ in MEM for 6 h at 37° C and then infecting with VSV at a multiplicity of 0.1. The inhibitor was maintained in the medium. After incubation for 19 h at 37° C VSV was collected from the extracellular fluids.

†This preparation of virus was identical to the preparation shown in Fig. 1. Compared with Fig. 1, the reduction in titre of the percentage of PFU resistant to neutralisation by anti-VSV serum was probably due to the disruption of HSV envelopes resulting from repeated freezing and thawing of the same virus preparation.

expression of herpes-like viruses as well as RNA tumour viruses. Also, although it is premature to conclude anything about the effect of pseudotype formation on viral oncogenesis, possible interactions of herpesviruses with RNA viruses at the level of host membranes may result in the rescue of defective herpesviruses by other enveloped viruses, and in the rescue of defective RNA tumour viruses by herpesvirus-coded surface antigens.

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types in which effects had been noted with polyoma virus. We report here that dexamethasone has different effects on HSV-2 infection *in vitro* depending on both the type of cell tested and the characteristics of the viral strain used.

The MS strain of HSV-2 was obtained from Dr L. W. Catalano and grown in our laboratory in Vero cell cultures in RPMI 1640 medium with 5% fetal bovine serum. Two sub-strains, one highly syncytia-forming (HSV-2syn) and the other predominantly non-syncytia-forming (HSV-2non-syn), were obtained by plaque purification from the uncloned virus which produced both syncytial and non-syncytial plaques on Vero cells.

Virus pools were grown in Vero cells and titrated by plaque assay with a methylcellulose overlay as before⁶. 3T3 cells were obtained from Dr George Todaro and Swiss mouse embryo fibroblasts (SMF) were obtained from Microbiological Associates. All experiments were performed with monolayer cultures in 25-cm² Flacon plastic flasks.

Dexamethasone (10^{-6} - 10^{-7} M) and cortisol (10^{-6} M Sigma) were prepared in absolute ethanol and dissolved to appropriate concentrations in medium or in the methylcellulose overlay. Media for control cultures contained ethanol in amounts equal to those in the steroid-containing media.

Monolayers of 3T3 cells were preincubated for 24 h in glucocorticoid-containing medium, infected with HSV-2 syn and fed with medium containing glucocorticoid, and the total amount of virus produced 24 h after infection was measured by plaque assay in Vero cell cultures⁶. A tenfold or greater increase in yield of virus was found in several experiments (Table 1).

Table 1 Effect of glucocorticoids on production and plaquing efficiency of HSV-2syn on 3T3 cells

Glucocorticoid	Viral yield (PFU per flask)	No. of plaques*
Dexamethasone 10^{-6} M	1.8×10^7	82
10^{-6} M	4×10^7	75
10^{-7} M	3×10^7	60
Cortisol 10^{-6} M	1×10^7	83
Control	5×10^5	16

Flasks containing 3×10^6 cells were infected with 10^6 PFU of HSV-2syn. The 24 h viral yield was measured after two cycles of freezing and thawing of cells and supernatant, followed by centrifugation at 1,000 r.p.m. for 10 min to remove cell debris, and then by plaque assay on Vero monolayers.

* Input 200 PFU as measured on Vero monolayers. Figures are averages of those for two replicate flasks.

To test whether the 3T3 monolayers had become more susceptible to HSV-2syn infection under the influence of the steroids, we studied the plaquing efficiency of the virus with a methylcellulose overlay containing steroids. Repeated experiments showed a twofold to fivefold increase in the number of plaques in the cultures treated with 10^{-6} - 10^{-7} M dexamethasone and 10^{-6} cortisol compared with ethanol controls (Table 1).

Because Thrash and Cunningham⁷ have shown that dexamethasone stimulates DNA synthesis and division of contact-inhibited 3T3 cells we measured the 24 h viral production of HSV-2syn in growing, non-confluent monolayers compared with contact-inhibited 3T3 confluent cell cultures. We found no significant differences in the yields. Thus the increase in viral production seems to be independent of the stimulation of division of contact-inhibited 3T3 cells by glucocorticoids.

In contrast to the results with HSV-2syn and 3T3 cells, when SMF monolayers were infected with HSV-2syn, steroids produced no change in 24 h viral production or plaquing efficiency. Dexamethasone and cortisol did, however, cause a decrease in the size of the plaques (Table 2). The reduction in plaque diameter correlated with a decrease in the ratio of released virus to cell-associated virus 24 h after infection (Table 3).

Since the spread of virus and plaque formation with HSV-

Effect of dexamethasone on herpes simplex virus type 2 infection *in vitro*

MORRHEN *et al.*¹ reported that when polyoma virus-infected cultures of mouse embryo fibroblasts and 3T3 cells were treated with physiological doses of corticosteroids, plaque formation was enhanced tenfold. Because the plaques appeared earlier and were larger in the presence of steroids, they suggested that the rate of production of virus was also increased. It has also been reported that glucocorticoids influence *in vitro* infections with murine leukaemia virus and mouse mammary tumour virus^{2,3}. Since there is epidemiological evidence that herpes simplex type 2 (HSV-2) can be oncogenic in man⁴ and evidence that inactivated HSV can produce malignant transformation *in vitro*⁵, we studied the effect of corticosteroids on HSV-2 infection in mouse embryo fibroblasts and 3T3 cells, the two cell

Table 2 Reduction in size of HSV-2syn plaques in SMF with glucocorticoid

Glucocorticoid	Plaque size (mm)
Dexamethasone 10^{-5} M	1.05
10^{-6} M	1.15
10^{-7} M	1.4
Control	2.4

Plaque diameter figures are averages of measurements of 10 plaques. The plaques were measured by a linear grid inserted in the ocular piece of a dissecting microscope.

2syn are related to both formation of polykaryocytes and release of virus from infected cells we investigated the effects of steroids on infection of SMF by non-syncytial HSV-2. We found no effects on virus yield, released fraction of virus, plaquing efficiency or plaque size. The reasons for this difference between the strains of HSV-2 are not clear, although steroids apparently affect the release of virus from polykaryocytes produced by HSV-2syn in SMF cultures but not from the mononuclear cells releasing HSV-2non-syn.

Table 3 Influence of glucocorticoids on the released fraction of HSV-2syn infecting SMF

Glucocorticoid	Viral yield (PFU per flask)*	R/CA ratio	
	Cell-associated (CA)	Released (R)	
Dexamethasone 10^{-5} M	28.8×10^5	80×10^5	2.7
10^{-6} M	28×10^5	70×10^5	2.5
10^{-7} M	36×10^5	15×10^5	0.4
Cortisol 10^{-6} M	18×10^5	45×10^5	2.5
Control	5×10^5	140×10^5	28.0

* Input 1×10^5 PFU per flask. Twenty-four hours after infection the supernatant of the cultures was collected and the virus present was titrated as 'released virus'. After removing the supernatant, 2 ml of medium was added to the culture flasks and the intercellular virus was recovered as described in the total viral yield experiments.

Our results show that the effect of glucocorticoids on HSV-2 infection *in vitro* depend on the type of cell culture as well as the characteristics of the viral strain. In 3T3 cells infected with the syncytial strain of HSV-2, steroids produce increased yields of virus and twofold to fivefold increases in plaquing efficiency. In Swiss mouse fibroblasts, steroids produce no increase in virus yield or plaquing efficiency, but there is a decrease in plaque size and amount of virus released from cells infected with the syncytial strain. The findings suggest that in HSV infections *in vivo*, in addition to effects on the immune system, steroids act directly on infected cells to either increase susceptibility and yield, or decrease release.

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Increased susceptibility to virus oncogenesis of congenitally thymus-deprived nude mice

A ROLE of thymus-deprived (T) lymphocytes in controlling tumour development has been widely discussed. The most convincing evidence in support of such a mechanism comes from observations on the induction of tumours by polyoma virus in adult mice¹. Normal adult mice do not develop tumours after infection with the virus, but mice deprived of T lymphocytes by thymectomy and repeated inoculations of anti-lymphocytic serum and infected with polyoma virus as adults do develop tumours. If such animals are reconstituted with T lymphocytes from syngeneic immune donors, tumours do not develop.

Congenitally thymus-deprived (*nu/nu*) mice provide a convenient test system for analysing the role of T lymphocytes in surveillance against tumours. These mice are unusually susceptible to infections by certain viruses and other agents¹, and they soon die if maintained in normal laboratory colony conditions. Nude mice can be maintained under special conditions for longer and those surviving for 7 months have not developed tumours spontaneously². The time of appearance and numbers of tumours in nude mice treated with methylcholanthrene were not significantly different from those in control mice³; the relatively large dose of carcinogen used could have been immunosuppressive, however, and the skin of a *nu/nu* mouse is clearly abnormal.

These observations have raised some doubts about the role of T lymphocytes in surveillance against tumours. We decided, therefore, to investigate the susceptibility of *nu/nu* mice to tumour induction by viruses. The *nu/nu* mice used were bred in this institute on a TO background; 20 male *nu/nu* mice and 20 littermate control *nu/+* mice aged 6-8 weeks were infected with polyoma virus. One half of the *nu/nu* and control animals were



Fig. 1 Nude mouse 8 weeks after infection with polyoma virus, showing multiple skin tumours.

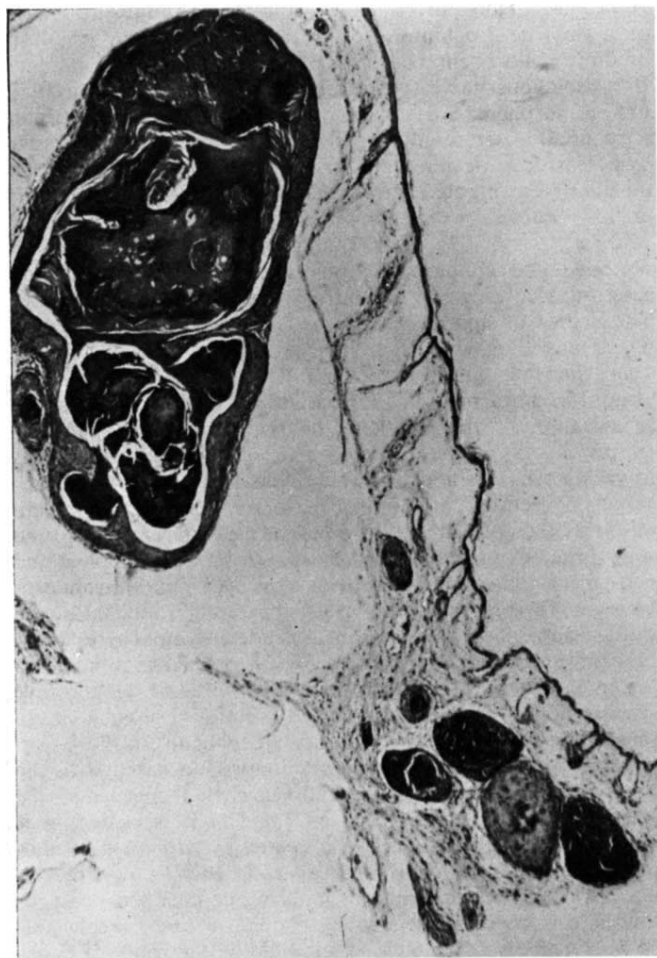


Fig. 2 Section of nude mouse skin, showing one large and several small tumours. ($\times 550$).

injected intraperitoneally with 0.2 ml horse anti-mouse lymphocyte serum (ALS) twice weekly for 8 weeks. No tumours developed in any *nu/+* mice. All *nu/nu* mice given ALS developed skin tumours within 4 weeks. Beginning 4 weeks after infection, untreated *nu/nu* mice developed skin tumours, and by 12 weeks all had multiple skin tumours (Fig. 1). The tumours were mostly carcinomas of the hair follicles (Fig. 2) and occasionally of sebaceous glands. Some mice also showed widely disseminated bone tumours, involving the femur, tibia, pelvic bones, vertebrae and ribs, as detected by radiological examination. The histological appearance was that of endosteal and periosteal osteosarcoma, the latter infiltrating muscle. Each animal had several bone tumours, mostly small but some as large as 1 cm in diameter. Other tumours present in the animals included adenocarcinomas of the salivary glands, carcinomas of the renal cortex and flat transitional-cell carcinomas of the renal pelvis.

In another experiment 6 male *nu/nu* mice and 12 *nu/+* littermate control mice aged 8 weeks were inoculated intra-

muscularly with 10^3 ID₅₀ of murine sarcoma virus (MSV/M). None of the heterozygous control animals developed tumours, whereas all *nu/nu* mice developed large tumours of their thighs and were killed 3 weeks later. They also had marked splenomegaly and histological examination showed lesions in the muscle and thighs expected of murine sarcoma virus⁴.

These results show that *nu/nu* mice are highly sensitive to virus oncogenesis at an age when their heterozygous littermates are resistant, confirming the major role of T lymphocytes in limiting virus oncogenesis. The accelerated development of tumours in recipients of ALS, however, suggests that there may be a second surveillance mechanism which does not involve T lymphocytes, possibly the antibody-dependent effector cell system⁵. This might account for the low incidence of tumours recorded in *nu/nu* mice², although they were observed only for 7 months.

MSV/M was provided by Dr J. J. Harvey.

Note added in proof: Prof. M. Vandeputte, Leuven, Belgium, has independently found that nude mice are highly susceptible to oncogenesis when infected with polyoma virus as adults.

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Effect of hypophysectomy on a virus-induced T-cell leukaemia

WE have found that removal of the pituitary from rats decreases their susceptibility to leukaemia induced by Gross virus. This provides preliminary support for our hypothesis that the pituitary through one of its hormones, is involved in leukaemogenesis.

Gross passage A virus was injected intraperitoneally into newborn Sprague-Dawley rats. At 3 weeks of age the animals were weaned and separated by sex, and approximately half of them were hypophysectomised. At weekly intervals thereafter they were weighed and then a differential and total white blood cell count was performed. Autopsies were conducted after death and leukaemia was diagnosed on the basis of gross and microscopic findings of the presence of typical lymphoblasts in the peripheral blood. Autopsied animals had thymus tumours and other pathology typical of T-cell leukaemia¹. Hypophysectomy was considered to have been complete if the animals gained less than 100% of their weight after surgery and the pituitary was absent at autopsy.

By 12 weeks after injection of the virus, the incidence of leukaemia was none out of 11 in hypophysectomised rats, 8 out of 12 in incompletely hypophysectomised rats, and 37 out of 37 in unoperated rats.

Thus hypophysectomy significantly impairs leukaemogenesis induced by Gross virus. It is not clear at the moment why our results conflict with those of Nagareda and Kaplan who found that hypophysectomy did not impair radiation-induced leukaemia in C57BL mice². In their study the T-cell lymphomas appeared slightly earlier in the operated animals.

The possibility that at least one phase of leukaemogenesis depends on the pituitary suggests new ways of investigating leukaemia in both experimental animals and man. In the case of

Table 1 Tumours induced in *nu/nu* mice inoculated with polyoma virus at 6–8 weeks of age

	% Tumours
Skin hair follicular carcinoma	100.0
Osteosarcoma	36.4
Salivary gland carcinoma	45.6
Carcinoma of kidney	55.6
Transitional cell carcinoma of renal pelvis	27.3

10^6 plaque-forming units of the small-plaque variant of SE polyoma virus inoculated intraperitoneally.

a T-cell lymphoma the pituitary may be affecting the thymic epithelium to create the conditions appropriate for leukaemogenesis. It may do this through known hormones, such as growth hormone or prolactin, or unknown hormones such as a thymus-stimulating hormone. Growth hormone which influences thymus development, may be the critical hormone³. If so, it probably acts by stimulating the synthesis of somatomedin, perhaps even a somatomedin unique for the thymus. If Gross virus impairs the synthesis of this somatomedin, leukaemia may develop as a consequence of the lack of a feedback inhibitor to suppress pituitary stimulation of the thymus.

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***In vitro* sensitisation to dinitrochlorobenzene in guinea pigs**

ACCORDING to Medawar's concept of peripheral sensitisation^{1,2} the induction phase of contact sensitivity may consist of a sequence of three stages. These are the formation of antigen by conjugation of the hapten with skin proteins; the recognition of this antigen by circulating lymphocytes and the activation of these cells passing by the site of antigen application; and the proliferation and differentiation of these activated lymphocytes in the draining lymph node resulting in development of memory and effector cells.

guinea pigs highly purified from granulocytes and macrophages on a glass bead column were incubated in a 10^{-5} M solution of dinitrochlorobenzene (DNCB) in medium 199 containing 10% isologous guinea-pig serum for 3 h at 37°C in a 10% CO₂ atmosphere. Suspension of 5×10^6 cells per ml medium were used. After removing as much free DNCB as possible by at least four washings, 3×10^7 – 5×10^7 lymphocytes in 0.2 ml medium were injected intradermally into the skin of the left ear of strain-13 recipients. These animals were skin-tested 14 d later with 0.09%, 0.05% and 0.03% DNCB solutions in acetone. The contact reactions were read 24 h later and assessed according to an arbitrary scale from 0 to 3 (ref. 4). The degree of hypersensitivity was expressed as the total of all three readings in each animal.

In the first group donors and recipients were normal strain-13 guinea pigs. In the second group the possible active sensitisation of the recipients by the hapten conjugated to lymphoid cells was excluded by using tolerant guinea pigs as recipients. Tolerance to DNCB was induced by two intravenous injections of dinitrobenzenesulphonic acid sodium salt (DNBSO₃) 28 d and 14 d before the transfer. It has been shown that tolerance induced by this method is complete and permanent and cannot be broken even by using dinitrofluorobenzene (DNFB) in FCA for sensitisation (group 5). In the third group, allogeneic peritoneal exudate lymphocytes from Himalayan spotted white guinea pigs were used as donor cells assuming that rejection of these cells will prevent the adoptive sensitisation of recipients having no effect on a possible active sensitisation with hapten-lymphocyte conjugate. In the fourth group, lymphocytes from tolerant donors incubated with the hapten were transferred to normal syngeneic recipients.

The results are summarised in Table 1. It is evident that peritoneal exudate lymphocytes treated *in vitro* with a non-toxic solution of DNCB are capable, when transferred to normal syngeneic recipients, of inducing contact sensitivity to this hapten, as demonstrated by a positive epicutaneous test 14 d after the transfer. This effect could be the result of either sensitisation of the lymphocytes *in vitro* or the formation of a strongly immunogenic antigen by conjugation of DNCB with lymphocytes, as shown by Baumgarten and Geczy⁵, Asherson

Table 1 Sensitisation of guinea pigs by intradermal injection of *in vitro* DNCB-treated lymphocytes

Group	Donors		Recipients		No.	Contact test	Sensitised/Total
	Strain	Immune status	Strain	Immune status			
1	13	Normal	13	Normal	6	1.6 ± 1.3	4/6
2	13	Normal	13	Tolerant	16	0.9 ± 0.7	12/16
3	Himalayan	Normal	13	Normal	5	0	0/5
4	13	Tolerant	13	Normal	7	1.4 ± 0.2	7/7
5	—	—	13	Tolerant* Controls	10	0	0/10

* Tolerant guinea pigs not receiving cells but injected with 500 µg DNFB in FCA and tested 14 d later.

Macher and Sommer³ failed to demonstrate convincingly peripheral sensitisation of lymphocytes in their combined *in vitro*-*in vivo* model using outbred guinea pigs, but their results do not exclude this concept either. Their main difficulty was to distinguish between active sensitisation with hapten-conjugated lymphocytes and transfer of sensitivity with cells already sensitised *in vitro*.

Here in our study, a similar model was used to solve this important problem. As tolerant guinea pigs cannot become sensitised by application of the hapten but are adoptively sensitised by cells from hypersensitive donors, immunocompetent lymphocytes, when activated *in vitro*, should also induce contact sensitivity in tolerant recipients.

Peritoneal exudate lymphocytes from inbred strain-13

*et al.*⁶ and McFarlin and Balfour⁷. This second possibility was excluded, however, in the experiments in groups 2 and 3.

It was demonstrated that guinea pigs rendered tolerant by two intravenous injections of DNBSO₃ cannot become actively sensitised even when a relatively high dose of hapten (500 µg DNFB) in FCA is used for sensitisation (group 5) and epicutaneous tests were repeated for a period of more than 1 yr. The tolerant animals become hypersensitive, however, by adoptive transfer of lymphoid cells from hypersensitive donors⁸.

It was shown recently⁹ that tolerance in contact sensitivity may be caused by specific suppressor cells preventing the proliferation of normal immunocompetent lymphocytes, probably at a very early stage. Our results in group 2, showing that 12 out of 16 tolerant recipients of lymphocytes preincubated

with DNCB *in vitro* became sensitised, indicate that these cells already reached a stage when they cannot be inhibited by the suppressor cells any more. These results favour the concept of *in vitro* sensitisation or at least activation of specific immunocompetent lymphocytes. The higher degree of sensitivity of normal recipients in comparison to tolerant ones might be caused by additional active sensitisation of normal animals, but not of tolerant ones, with a conjugate of DNCB with lymphoid cells⁶⁻⁷.

The idea that lymphocytes can actually be sensitised *in vitro* is further supported by the finding that allogeneic cells (group 3) as well as non-viable autologous cells⁸ have no sensitising capacity, although their capacity to form immunogenic conjugates may not be altered.

The results in group 4 do not necessarily contradict the hypothesis of peripheral sensitisation; they need, however, a further explanation. One possibility is that the lymphocyte suspension from tolerant donors may either not contain suppressor cells or these cells are not able to act *in vitro* and prevent immunocompetent cells from becoming activated by preincubation with the hapten.

As it is known that guinea pigs can become sensitised to DNCB conjugated to different homologous skin and serum proteins¹⁰, and therefore also to DNP-conjugates formed with proteins of the culture medium, the present results might be regarded as evidence that the first two steps of the induction phase of contact sensitivity, namely formation of the antigen and its recognition by immunocompetent cells, can be accomplished also *in vitro*. Lymphocytes incubated with DNCB *in vitro* become activated beyond a stage where they cannot be inhibited any more by suppressor cells. An intradermal injection of these cells acts somehow as an adoptive sensitisation. An additional active sensitising effect of a DNCB-lymphocyte conjugate, however, may not be excluded when normal recipients are used. This may explain the higher degree of sensitisation in normal recipients than in tolerant ones.

As the sensitisation of tolerant recipients can be achieved only by transfer of already specifically activated lymphocytes, we assume that in our experiments cells incubated with the hapten *in vitro* were actually sensitised. The fact that in our *in vitro-in vivo* model the initial steps of sensitisation are accomplished *in vitro* may be regarded as support for the concept of peripheral sensitisation.

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Large numbers of cells in normal mice produce antibody components of isologous erythrocytes

THERE are a number of arguments against the idea¹ that self-tolerance depends on a purge of all self-reactive clones early in development. First, antigens which appear later in development, for example, Ig idiotypes, must induce tolerance to themselves. Second, autoimmune diseases are often reversible, implying that when anti-self-reactive cells arise late, they may still be controlled. Third, the recent finding (refs 2-4 and L. M. Pilarski and A.J.C., unpublished) that single clones of antibody-forming cells rapidly produce variants also implies a continuous monitoring and control of immunocompetent cells. Self-reactive variants must arise during many immune responses, and must normally be prevented from causing autoimmune disease. Fourth, the distinction between anti-self and anti-not-self specificities is obviously not clear-cut. Given the great diversity of self components in mammals, it is probable that almost any antibody will react to some self antigens with low affinity.

These considerations suggest that normal animals may contain many auto-reactive cells which are, however, prevented from causing autoimmune disease by some control mechanism. Here I demonstrate plaque forming cells (PFCs) against components of autologous erythrocytes (RBCs) in normal mice.

Although others have reported the presence of small numbers of plaques on normal isologous RBCs⁵⁻⁷, I could not find such PFCs in spleens of 8-week-old CBA mice. When their RBCs were first treated with any of the proteolytic enzymes trypsin, papain or bromelain, however, several thousand PFCs were found per spleen in normal mice of this and other strains (Table 1). Bromelain was used throughout the rest of this study: it is an enzyme which has been fairly widely used⁸⁻¹² to demonstrate allo- and autoantibodies against human RBCs. The mouse RBCs were first incubated as a 50% suspension with bromelain at a final concentration of 10 mg ml⁻¹ in phosphate buffered saline for 30 min. The RBCs were washed three times and then incubated at 37°C for 1 h with spleen cells in slide assay chambers¹³. Rabbit serum as a complement source gave about three times as many, and larger, plaques than normal guinea-pig serum. Only direct plaques were found—a guinea-pig anti-mouse Ig failed to increase the number of plaques at any dilution. They were up to about 0.5 mm in diameter, with many very small plaques.

A number of control experiments confirmed that these plaques were caused by release of Ig from a single central cell. In summary: the plaques did not appear in the absence of complement, or at 4°C, and they were inhibited by guinea-pig and anti-mouse-Ig antiserum to exactly the same extent as mouse anti-sheep RBC plaques, over a range of concentrations of antiserum. At high dilutions of spleen cells a single central cell could be seen in each plaque, and six of these PFCs, when isolated by micromanipulation¹⁴ and stained looked like the basophilic mononuclears which make conventional anti-sheep RBC plaques¹⁵. Plaques were not affected by preabsorbing the rabbit complement with bromelain-treated mouse RBCs, showing that they were not caused by a reaction between secreted mouse protein and a cytophilic rabbit-anti-mouse antibody on the treated red cells. They were not caused by nonspecific (Fc) adherence of secreted Ig to treated RBCs as immunisation with sheep RBCs which produced approximately 10⁵ anti-sheep PFCs per spleen, did not increase the number of plaques on bromelain-treated mouse RBCs. Also high concentrations of normal mouse serum in the assay mixture did not inhibit plaque formation.

Table 1 Numbers of plaques against bromelain-treated CBA mouse RBCs in various lymphoid tissues

Species and strain of animal	Age	No. of animals tested	Tissue	PFCs per 10 ⁸ nucleated cells arithmetic mean (+ range)
CBA mice*	8-10 weeks	42	Spleen†	5,320 (960-15,840)
CBA mice‡	8-10 weeks	4	Spleen	1,840 (1,280-2,480)
C57BL	8-10 weeks	4	Spleen	940 (480-1,280)
BALB/c	8-10 weeks	4	Spleen	1,020 (880-1,120)
(CBA × C57BL) F ₁	8-10 weeks	4	Spleen	2,200 (640-3,440)
NZB	8-10 weeks	4	Spleen	4,900 (1,440-10,400)
Nude (nu/nu)	8-10 weeks	4	Spleen	560 (80-1,520)
CBA mouse§	8-10 weeks	3	Bone marrow	2,600
			Thymus	100
			Mesenteric lymph node	600
CBA mouse	5-6 d	6	Spleen	33 (15-70)
	9-11 d	8	Spleen	229 (30-900)
	21 d	6	Spleen	2,317 (600-5,200)
	6 months	3	Spleen	2,400 (1,120-4,000)
Lewis rat	3 months	5	Spleen	1,560 (750-2,540)
BALB/c mice¶	8 weeks	6	Spleen	1,400 (680-1,920)
CBA mice**	8-10 weeks	4	Spleen	206,000 (147,000-272,000)

* This is a summary of many separate experiments.

† Plaques were counted in a fraction of a spleen cell suspension (usually 1/40). The total number of cells per adult mouse spleen has been assumed to be 10⁸ throughout this table.

‡ These four mice, and the mice of five other strains below it, were all assayed on the same day against the same preparation of bromelain-treated mouse RBCs. The sensitivity of the assay appeared to vary from day to day, and was low on this occasion. There was no significant difference between plaque numbers on isologous or allogeneic treated RBCs.

§ A pool of tissues from three 8-week-old mice was tested in each case.

|| In a separate experiment, three 6-month-old NZB females had 9,100, 12,000 and 22,400 PFCs per spleen.

¶ These mice were taken from strictly isolated germ-free conditions.

** Assayed 2 d after 50 µg LPS (intraperitoneal injection).

CBA mice were treated in various ways to try and increase the number of PFCs in their spleens against isologous erythrocytes which had been incubated with bromelain. The following treatments did not increase the number of plaques significantly over a period of one week: injection of allogeneic red cells or spleen cells, injection of isologous cells in Freund's complete adjuvant, a single injection of 10⁹ rat RBCs. A dramatic rise in the number of plaques was, however, induced by a single intraperitoneal injection of 50 µg of lipopolysaccharide (LPS) from *Escherichia coli* strain 0128:B12 (Table 1). These plaques peaked at 2-3 d then returned abruptly to normal background levels (in preparation).

Against what antigens are these antibodies directed? There are two broad possibilities: either they react with entirely new antigenic determinants created by the enzyme treatment, or they are directed against antigens which are present within the normal RBC. The first alternative seems

to be ruled out by two findings. First, bromelain-treated mouse RBCs provoke little or no increase in the number of plaques on similar target cells when injected into mice (Table 2). In other words, the enzyme treatment has not converted mouse RBCs into grossly foreign particles. Second, rats injected with normal mouse red cells respond by producing both increased numbers of PFCs (Table 2) and high antibody titres (Table 3) against bromelain-treated mouse RBCs. This shows that the antigens on the surfaces, of such treated cells do form part of the normal mouse RBC.

What does the bromelain treatment do to the RBCs to allow mouse lymphoid cells to cause their lysis? Again there seem to be two possibilities. The enzyme may make the RBCs more fragile and able to be lysed by lower affinity, or fewer, autoantibodies. Alternatively, the bromelain may expose some component(s) normally not available to the antibody, which are, however, revealed during normal

Table 2 Numbers of PFCs in the spleens of mice and rats injected with normal or bromelain-treated mouse RBCs

Experiment No.	Test animals species (+No.)	RBCs injected	Days after injection	PFCs per spleen (±s.e.) against BRBCs*	PFCs per spleen (±s.e.) against NRBCs*
1	CBA mice (3)	3 × 10 ⁸ BRBCs*	4†	4,056 ± 80	NT‡
	CBA mice	3 × 10 ⁸ BRBCs	4	4,064 ± 928	NT
	CBA mice	3 × 10 ⁸ NRBCs	4	4,076 ± 557	NT
2	CBA mice (3)	2 × 10 ⁹ BRBCs	3	9,812 ± 1,768	NT
	CBA mice	2 × 10 ⁹ NRBCs	3	2,094 ± 346	NT
	CBA mice	2 × 10 ⁹ BRBCs	6	4,108 ± 504	NT
	CBA mice	2 × 10 ⁹ NRBCs	6	2,508 ± 509	NT
3	Lewis rat No. 1	6 × 10 ⁸ NRBCs	4	41,600	89,600
	Lewis rat No. 2	6 × 10 ⁸ NRBCs	4	19,680	32,000
	Lewis rat No. 3	6 × 10 ⁸ NRBCs	4	66,560	126,720

In experiment 1, RBCs were injected intravenously, and in experiments 2 and 3, intraperitoneally.

* BRBC, Bromelain-treated CBA mouse RBC; NRBC, normal CBA mouse RBC.

† In this experiment there was also no significant response on days 1, 2 or 7 after injection.

‡ NT, Not tested. In similar experiments where spleens from normal mice or mice immunised with normal or bromelain-treated RBCs were tested, no plaques were found on normal target RBCs.

breakdown of the erythrocytes either in foreign species (rats) or in mice. This latter explanation is supported by results of absorption of serum from rats immunised with normal mouse RBCs (Table 3). Absorption with bromelain-treated mouse RBCs preferentially lowered the titre to similarly treated target RBCs without much affecting the titre to normal mouse RBCs, and vice versa. This seems to show that anti-bromelain-mouse RBCs and anti-normal-mouse RBC antibodies are different populations. Similarly, rats immunised with normal mouse RBCs had more plaques on normal than on bromelain-treated mouse RBCs, again showing that the treated cells are not merely a more sensitive indicator of all antibody which lyses normal cells; they seem to have different antigenic surfaces.

Attempts to demonstrate plaques on isologous RBCs in chicken and sheep have yielded none so far with either normal or bromelain-treated RBCs. Normal rats had less than 30 PFCs per spleen on treated or untreated rat RBCs, but approximately 8,000 PFCs were found in the spleens of LPS-stimulated rats tested against bromelain-treated rat RBCs. It is interesting that rats do have a high normal plaque level to bromelain-treated mouse RBCs (Table 1): possibly mouse and rat RBCs share common internal antigens which are more difficult to reveal by enzyme treatment of the rat RBCs.

The numbers of plaques against bromelain-treated isologous RBCs in the spleens of normal mice is more than an order of magnitude higher than the 'background' level described by many authors against highly immunogenic foreign erythrocytes. This suggests an ongoing response to breakdown products of autologous RBCs, producing antibody which may assist removal of effete cells. The idea that the antigen comes from within rather than from cross-reactive environmental stimuli is reinforced by the fact that germ-free mice had similar plaques (Table 1).

Table 3 Absorption of rat-anti-mouse RBC serum with normal or bromelain-treated mouse RBCs

Serum pool	Days after injection*	Absorbed with†	Titre‡ against mouse RBCs	
			Normal	Bromelain-treated
1	4	—	256	256
		Normal RBCs	<8	256
		Bromelain RBCs	128	<8
2	6	—	1,024	4,048
		Normal RBCs	32	760
		Bromelain RBCs	256	32
3	7	—	128	512
		Normal RBCs	<8	256
		Bromelain RBCs	128	<8

* Each serum is from a pool of three Lewis rats injected intraperitoneally with mouse RBCs. Antigen dose was 6×10^8 RBCs for sera 1 and 3, and 2×10^8 for serum 2.

† Absorptions were done by mixing 0.5 ml of serum, diluted 1:8, with 0.2 ml of normal or bromelain-treated mouse RBCs. The mixture was incubated for 15 min at 37°C, then the RBCs removed by centrifugation. This was repeated twice.

‡ Titres are reciprocals of 50% haemolytic end point, using rabbit complement (final dilution 1:20) and RBCs at a final dilution of 0.5%.

This work raises two further questions about tolerance to autologous erythrocytes. First, why is tolerance to the surface of untreated RBCs so much more 'complete', in that relatively few plaques can be found on such targets? This may depend on an earlier confrontation between 'external' RBC antigens and the developing host immune system. The life span of mouse RBCs is 20–45 d (ref. 16), so large numbers of degenerating cells might not be available to act as antigen until 1–2 weeks after birth, while intact RBCs would be present much earlier. Second, why do plaque

numbers stabilise at about 5,000 per spleen (in adult CBA mice) when there is presumably ample antigen available, as RBCs degenerate, to push the response still higher? Even large doses of bromelain-treated RBCs will not increase this number much (Table 2). It seems possible that an equilibrium is maintained between constant antigen stimulation and some suppressor mechanism. This view, which has important implications for self-tolerance, is supported by recent experiments (data in preparation) showing that injection of normal mice with antilymphocyte serum will increase the numbers of plaques in their spleens against bromelain-treated isologous RBCs.

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Note added in proof: Since submitting this paper I have read the article by Detleer and Edginton (*Clin. exp. Immunol.*, **16**, 431; 1974) which describes similar numbers of PFC on bromelain-treated mouse erythrocytes from NZB mice.

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Inhibition of rat lymph node cell cytotoxicity by hepatoma-associated embryonic antigen

ONE feature frequently accompanying malignant transformation is the re-expression of embryonic characteristics, and the presence of tumour-associated embryonic antigens has been demonstrated on a wide range of experimental and human tumours^{1–5}. Studies in our laboratory, using aminazo-dye-induced rat hepatomas, have shown that these re-expressed antigens are detectable on cells derived from 14–16-d-old rat embryos, but not cells from adult rat tissue^{1,6} and *in vitro* microcytotoxicity tests have revealed that rats bearing transplanted hepatomas exhibit a cell-mediated immune reaction against the embryonic antigen (EA)⁶.

Here we present results which demonstrate that embryonic antigen isolated from two rat hepatomas is capable of inhibiting the cytotoxicity of lymphocytes specifically sensitised to

Table 1 Effect of hepatoma D23 associated embryonic antigen on the *in vitro* cytotoxicity of multiparous and tumour-immune rat lymph node cells

Test	Target cells	Status of LNC donor	LNC pretreated with D23 EA*	Mean no. target cells surviving per well ($\pm$ s.e.) after exposure to:		% Lymphocyte cytotoxicity	% Inhibition
				Normal LNC	Test LNC		
1	D23	Multiparous	—	28 $\pm$ 4	7 $\pm$ 1	75¶	—
			12.5	31 $\pm$ 5	30 $\pm$ 2	3	96§
		D23-immune	—	28 $\pm$ 4	9 $\pm$ 2	68¶	—
			12.5	31 $\pm$ 5	7 $\pm$ 1	77¶	-13
2	D23	Multiparous	—	78 $\pm$ 1	51 $\pm$ 7	35‡	—
			5	73 $\pm$ 8	59 $\pm$ 5	19	46
		D23-immune	10	64 $\pm$ 7	60 $\pm$ 6	7	80‡
			—	78 $\pm$ 1	59 $\pm$ 8	24‡	—
3	D23	Multiparous	—	22 $\pm$ 2	9 $\pm$ 1	59¶	—
			15	23 $\pm$ 2	11 $\pm$ 1	52¶	12
		D23-immune	30	22 $\pm$ 2	17 $\pm$ 1	23‡	61‡
			—	22 $\pm$ 2	6 $\pm$ 1	73¶	—
4	D23	Multiparous	—	22 $\pm$ 2	6 $\pm$ 1	73¶	—
			15	23 $\pm$ 2	8 $\pm$ 1	65¶	11
		D23-immune	30	22 $\pm$ 2	11 $\pm$ 3	50§	32‡
			—	26 $\pm$ 2	12 $\pm$ 2	54¶	—
5	D23	Multiparous	—	26 $\pm$ 2	12 $\pm$ 1	54¶	—
			15	26 $\pm$ 2	12 $\pm$ 1	54¶	0
		D23-immune	30	32 $\pm$ 4	23 $\pm$ 5	28	48‡
			—	25 $\pm$ 1	17 $\pm$ 3	32‡	—
6	14-d embryo	Multiparous	—	26 $\pm$ 3	16 $\pm$ 2	38‡	-19
			15	26 $\pm$ 2	26 $\pm$ 3	0	100‡
		D23-immune	30	26 $\pm$ 2	26 $\pm$ 3	0	100‡
			—	26 $\pm$ 2	26 $\pm$ 3	0	100‡

* Hepatoma D23 associated embryonic antigen added per 5×10^5 LNC (μ g protein). † $P = < 0.1$ ‡ $P = < 0.05$
 § $P = < 0.01$ ¶ $P = < 0.001$.

embryonic antigen component against hepatoma cells *in vitro*. This mechanism is similar to that shown for the individually distinct hepatoma-specific antigen⁸. The relevance of our findings to the modification of tumour growth *in vivo*, and to antigenic expression on human tumours is also discussed.

Our investigation was designed to evaluate whether in a model *in vitro* system, hepatoma-associated EA had the capacity to interfere with lymphocytotoxic reactions. For this purpose, EAs were isolated following sequential fractionation of the soluble cytoplasmic protein fraction of homogenates from two transplanted hepatomas (D23 and D30) as previously described^{1,7}. These procedures make possible the recovery of an antigenic fraction which by conventional criteria seems homogeneous although most recent studies indicate that this material represents at least two distinct, but co-purifying, macromolecules; one being a protein component of molecular weight between 65,000 and 70,000 and the other being a glycoprotein of lower molecular weight (M.R.P., and Z. Tökés, unpublished). The

final EA preparations from hepatomas D23 and D30 retain the capacity to neutralise the membrane immunofluorescence staining of viable tumour cells by multiparous (MP) rat sera which have been shown to contain antibody directed against cell surface-expressed EA^{1,7}.

Hepatoma D23 and D33 cells were cultured as monolayers in Eagle's minimal essential medium (MEM)+10% calf serum, and cells obtained following trypsinisation of whole 14-d-old rat embryos were grown in Waymouth's medium+10% foetal calf serum. For lymphocytotoxicity tests, tumour or embryo cells were plated in the wells of Microtest II plates (Falcon) at a concentration of 100–200 cells per well in Eagle's MEM+10% calf serum, and incubated for 24 h at 37°C to allow adherence. MP lymph node cells (LNC), obtained from the cervical, axillary and mesenteric lymph nodes were taken from rats having had three or more pregnancies and which were pregnant at the time of assay. Tumour-immune LNC were obtained from rats which had received three or more γ -irradiated (15,000 rad)

Table 2 Effect of hepatoma D30 associated embryonic antigen on the *in vitro* cytotoxicity of multiparous rat lymph node cells

Test	Target cells	LNC pretreated with D30 EA*	Mean no. target cells surviving per well ($\pm$ s.e.) after exposure to:		% Lymphocyte cytotoxicity	% Inhibition
			Normal LNC	MP LNC		
1	D23	—	33 $\pm$ 4	16 $\pm$ 3	52‡	—
		20	40 $\pm$ 6	21 $\pm$ 4	48‡	8
		40	39 $\pm$ 4	32 $\pm$ 5	18	63‡
2	D23	—	41 $\pm$ 6	29 $\pm$ 2	29‡	—
		50	42 $\pm$ 6	42 $\pm$ 5	-2	107‡
3	D33	—	350 $\pm$ 51	38 $\pm$ 9	89§	—
		50	384 $\pm$ 49	86 $\pm$ 15	78§	12
4	D33	—	350 $\pm$ 51	55 $\pm$ 10	84§	—
		50	384 $\pm$ 49	119 $\pm$ 32	69§	18

* Hepatoma D30 associated embryonic antigen added per 5×10^5 LNC (μ g protein). † $P = < 0.05$ ‡ $P = < 0.01$ § $P = < 0.001$

hepatoma D23 grafts, followed by challenge with 5×10^5 viable tumour cells at monthly intervals. Aliquots (0.4 ml) containing 5×10^6 LNC were incubated at 37°C for 60 min with 0.2 ml volumes of EA at various concentrations or in control tests, with medium alone (0.2 ml aliquots MEM). LNC were sedimented at 120g for 5 min and suspended in 2 ml MEM. 5×10^5 LNC (0.2 ml volume) were added to each of eight wells in Microtest II plates and after 60 min foetal calf serum was added to a concentration of 10% in each well. After incubation for 48 h at 37°C any remaining adherent tumour cells were washed, fixed, stained with Gentian violet and counted.

Table 1 (tests 1–5) illustrates the effect of pretreating MP or D23-immune LNC with hepatoma D23 EA. Previous incubation of 5×10^6 MP LNC with 12.5 μg D23 EA reduced their cytotoxicity from 75 to 3%, representing a 96% inhibition of cytotoxicity (Table 1, test 1). This concentration did not, however, reduce the cytotoxic effect shown by LNC from hepatoma D23-immune animals for plated hepatoma cells (Table 1, test 1). Comparable results were obtained in a subsequent experiment (Table 1, test 2) again indicating that D23 EA, at concentrations which inhibit MP LNC cytotoxicity does not affect the cytotoxicity of D23-immune LNC. These findings give further evidence demonstrating that the tumour-specific antigen, present on these aminoazo dye-induced rat tumours and against which tumour-immune LNC exert their cytotoxic effect, is a distinct component from the tumour-associated EA. Tests 3–5 (Table 1) exemplify further data showing the inhibitory effect of D23 EA on the cytotoxicity of MP LNC, and in test 6 (Table 1) the cytotoxicity of MP LNC for 14-d rat embryo cells is also reduced following previous exposure to hepatoma D23 EA.

In comparable tests, the EA preparation from another transplanted hepatoma (D30) was found to display similar activity to the EA isolated from hepatoma D23 (Table 2). Pretreatment of 5×10^5 MP LNC with 40–50 μg of hepatoma D30 EA significantly inhibited their cytotoxicity for D23 target cells (Table 2, tests 1 and 2). This finding, indicative of a cross-reactive nature between hepatoma D23 and D30 EA, is also supported by previous studies showing that the isolated EA preparations had the capacity to neutralise the membrane immunofluorescence staining by MP sera of surface antigens expressed on either D23 or D30 target cells^{1,7}. A variability in EA expression or specificity, however, was indicated in further experiments using MP LNC pretreated with hepatoma D30 EA (Table 2, tests 3 and 4). In these tests, the cytotoxicity of MP LNC for plated hepatoma D33 cells was not significantly reduced by previous exposure to D30 EA at a concentration of 50 μg per 5×10^5 LNC.

Previous studies have established the existence of two distinct neoantigens expressed at the cell surface of chemically-induced hepatomas. These antigens have been differentiated into a tumour-specific component and an embryonic component by several criteria^{1,9}. Thus, MP rat serum was shown to block, at the level of the target cell, MP LNC cytotoxicity for hepatoma cells, but was not capable of blocking tumour-immune LNC cytotoxicity for target hepatoma cells. The reactivity of tumour-immune rat LNC for 14 d embryo cells was, however, effectively prevented by MP serum. These results suggest that the host's cellular immune response is manifested by two separate populations of sensitised lymphocytes. In our study a similar differentiation between the two neoantigens is shown; MP LNC cytotoxicity for hepatoma cells was inhibited at the effector cell level by hepatoma-associated EA, but this treatment did not affect the cytotoxicity of tumour-immune LNC for hepatoma cells. The involvement of hepatoma-associated EA in eliciting a tumour-rejection response is less clear. Immunisation of rats with foetal tissue failed to induce protection against low dose hepatoma cell challenge¹⁰. Similar results have been found using other tumour systems^{11,12}, although embryo immunisation has been shown to induce protection against SV40-induced hamster tumours^{13,14} and 3-methylcholanthrene-induced mouse, rat

and guinea pig sarcomas^{15–17}. The failure of embryonic antigens to induce an effective tumour-rejection reaction may be due to the stability, and/or transient appearance of the antigen on the embryo cell surface^{1,3,13}; alternatively the route of administration together with the immunising regime and effective EA dosage may greatly influence the eventual immunological response.

Our findings are relevant to studies on human tumours as the embryonic antigens detected by microcytotoxicity tests on carcinogen-induced rat mammary⁶ and colonic carcinomas¹⁸ as well as spontaneously arising rat mammary carcinomas and sarcomas⁶ show organ-type specificities comparable with those identified on many human tumours¹⁹ and by analogy the latter may also be re-expressed embryonic antigens. It has further been demonstrated with a limited number of human tumours that antigen mediated inhibition of lymphocyte cytotoxicity may be one mechanism by which a growing tumour evades host immunological control^{20,21}. Recent studies showing the presence of hepatoma-associated EA in the serum of tumour-bearer rats implies that such factors may be capable of modifying lymphocytotoxic reactions *in vivo* (R.C.R., M.R.P., R.W.B., and L.P.S., unpublished), as demonstrated here using *in vitro* tests.

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Association of mammalian cell death with a specific endonucleolytic degradation of DNA

MAMMALIAN cell death, observed as deterioration of cellular structure or function, has not been characterised at the molecular level. We report here that human and rodent cells treated with several diverse agents which cause cell death as measured by other criteria, had similar distinct patterns of DNA degradation as assayed by alkaline sucrose gradient techniques. The degradation results from cellular metabolism, not from the initial damage produced by the agents. The similarity of the patterns in our results and those of others in a wide range of cell systems suggests that this degradation may be a general mechanism concomitant with, if not a precedent of, mammalian cell death.

Tritiated thymidine (TdR) is a toxic agent when incorporated into the DNA of mammalian cells^{1,2}. The soft β radiation of tritium disintegration has a range of about 1 μ m and damages primarily the DNA and its immediate molecular environment. The time course of this toxic action as indicated by cell growth kinetics in monolayers of Chang's liver cells (LICH)³ and Chinese hamster lung cells (CHL)⁴ is shown in Fig. 1. As the growth rate of monolayer cultures thus treated with ³H-TdR slowed down and the cell density eventually declined, intact cells floated into the medium above the attached cells and could be recovered by centrifugation. These floating cells, though normal in appearance by phase microscopy, had a plating efficiency approximately 1% of that of attached cells. After 24 h of exposure to ³H-TdR, LICH cells floating in the medium incorporated only 13% of ¹⁴C-leucine and 32% of ¹⁴C-uridine compared with attached cells for a 2-h pulse.

Sedimentation profiles of DNA from floating and attached cells differed (Fig. 2b). The latter was similar to the profiles reported for diploid and aneuploid cell lines⁵⁻¹³, and identical to control profiles of LICH and CHL cells (Fig. 2a and e). They consist of a unimodal homodisperse peak estimated by various authors to be between 125S and 180S⁵⁻⁹ which, if composed of single-stranded molecules, would correspond to a weight of between 1×10^8 and 5×10^8 .

The DNA profile of the floating cells, however, was bimodal with a smaller peak between fractions 4 and 6 (~50S) (Fig. 2b). The amount of DNA found in the degraded peak varied with time and metabolic activity. Identical cultures of LICH cells were labelled for 24 h with 0.5 μ Ci ml⁻¹ ³H-TdR and then incubated in either complete medium (CM) or phosphate buffered saline (PBS) for 8 h. This PBS treatment does

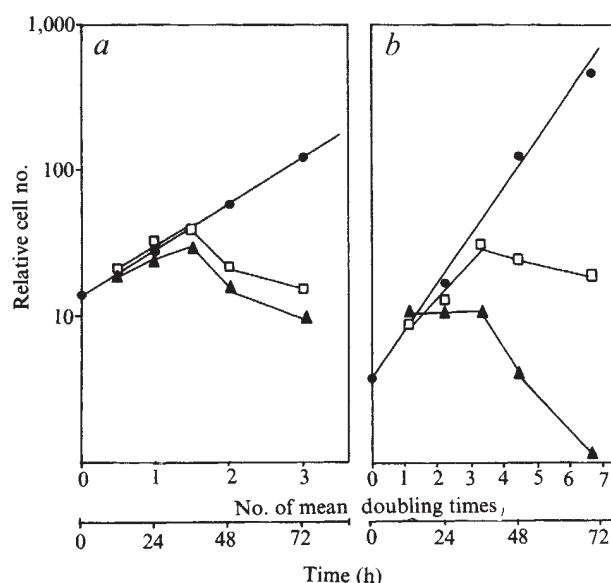


Fig. 1 Relative number of LICH (a) and CHL (b) cells growing in attached monolayers after continued exposure to 0.5 (□) or 5.0 (▲) μ Ci ml⁻¹ of tritiated thymidine (methyl-³H-TdR, 6-15 Ci mmol⁻¹) in Eagle's minimum essential medium supplemented with 10% heat inactivated calf serum (LICH) or 15% foetal calf serum (CHL). At times indicated cells were suspended using trypsin and counted using a haemocytometer. ●, Control.

not produce toxicity or degraded DNA. Cells were then exposed to fresh medium and incubated at 37° C. DNA sedimentation profiles of the floating cells appearing in these cultures were determined 24 and 48 h later. At both times, the DNA of floating cells from cultures whose metabolism was suppressed for 8 h with PBS was less degraded than those incubated in complete medium (Fig. 2c and d). In both cases, however, degradation was more extensive after 48 h than 24 h.

Treatment with toxic agents whose initial action is believed to affect the plasma membrane produced DNA degradation in CHL monolayer cultures. Attached CHL cells, labelled at low ³H-TdR levels which do not affect growth kinetics, then treated with hypotonic shock, 0.5% deoxycholate or specific CHL antiserum, displayed degradative patterns similar to those induced by β radiation but after shorter post-treatment incubation (Fig. 2f-h). After 5 min of hypotonic shock or deoxycholate treatment, immediate lysis showed the DNA of treated cells was intact, but incubation at 37° C in

Table 1 Observation of degraded DNA after trauma

Cells	Nature of insult	Presumed site of initial insult	Time after insult degradation observed (h)	Reference
LICH	³ H-decay	DNA	24	This report
LICH	³ H-decay	DNA	24	14
CHL	Specific antibody	Cell membrane	0.5	10
CHL	Specific antibody	Cell membrane	1	This report
CHL	Hypotonic shock	Cell membrane	1	This report
CHL	Detergent	Cell membrane	1	This report
CHL nuclei	Nuclei isolation	Nucleus	0.5	Nagle and Belli (unpublished data)
Progeric fibroblasts	³ H-decay	DNA	4	11
Human lymphocytes	External X rays	Cell	0.5	12
Beagle neurones	External X rays	Cell	10	5
Rabbit retina	External X rays	Cell	10	6
Mouse thymocytes	External X rays	Cell	0.75 (in vitro)	13
			3.5 (in vivo)	

complete medium for 60 min and 55 min, respectively, produced extensive degradation (Fig. 2f and g). Similarly, the DNA of cells treated for 60 min at 37°C with medium containing a 1:500 dilution of specific CHL antiserum after the method of Shipley *et al.*¹⁰ was also degraded into similar patterns.

Patterns of DNA degradation similar to those in Fig. 2 have been observed in various mammalian cell populations exposed to several toxic agents. Table 1 lists observations of DNA degradation in experimental systems described here and similar patterns observed by others. Several important conclusions can be drawn: (1) DNA is degraded in both diploid and aneuploid cells after trauma; (2) the decay of ³H-TdR is not necessary for degradation since Lett *et al.*⁵ and Wheeler *et al.*⁶ used non-dividing, unlabelled diploid cells;

(3) since Nagle and Belli (unpublished data) observed abrupt DNA degradation in isolated CHL nuclei with intact DNA by adding only ATP, the endonucleases need not be induced but may be constituent to the nucleus; (4) in human lymphocytes which are known to be especially sensitive to X rays, DNA appears to be degraded earlier¹² than in cells considered to be less sensitive, such as LICH or diploid CNS cells of the rabbit⁶ and dog⁵, and (5) in all cases, cellular metabolism was required to produce degradation.

It seems clear that similar patterns of degradation are common to various mammalian cells after DNA damage, cell membrane perturbation or general toxicity. The degraded DNA in every case had a modal value of between 10⁶ and 10⁷ daltons. Furthermore, since there is little radioactive label in the first two gradient fractions where mononucleotides and oligonucleotides would sediment, degradation must have resulted from an endonucleolytic incision as opposed to exonucleolytic attack which would produce these small fragments. Our results do not indicate whether the incisional attack on the DNA molecule was at random or rather at particular sites that have structural or functional meaning.

The observation of similar patterns of DNA degradation in cells exposed to widely differing types of trauma suggests that this process is a common mechanism associated with the phenomenon of cell death. As is the case for organisms, cells damaged beyond their recuperative capacity may undergo a distinct, non-reversible process which ends coherent metabolism.

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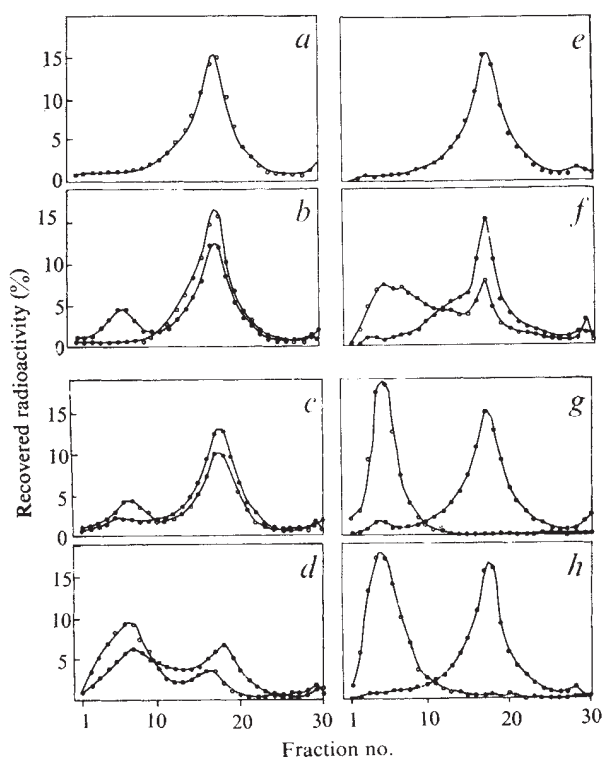


Fig. 2 DNA sedimentation patterns for cells which have received treatment with various toxic agents with and without post-treatment incubation. Cells attached to glass or plastic Petri dishes were labelled with tritiated thymidine (methyl-³H-TdR, 6-15 Ci mmol⁻¹, New England Nuclear Corp.), and either treated in monolayer or after suspension by trypsin treatment. After suspension, 2,500-10,000 cells were carefully layered into a lysing solution (0.2 ml, 0.45 N NaOH, 0.5 N NaCl, 0.1 M Na₂EDTA) on top of preformed 5-20% alkaline sucrose gradients (4.8 ml, 0.1 N NaOH, 0.9 N NaCl, 0.003 M Na₂EDTA). After lysing for 4-6 h samples were centrifuged for 15 min at 5,000 r.p.m. followed by 36,000 r.p.m. for 60 min (Spinco 50.1 rotor) at 12-15°C. Gradients were pumped off from the top in 10-drop fractions on to 30 glass fibre filters and counted in a toluene-base scintillation fluid. *a-d*, LICH cells: *a*, control LICH cells incubated with 0.1 μCi ml⁻¹ ³H-TdR for 16 h; *b-d*, LICH cells incubated with 5.0 μCi ml⁻¹ for 24 h; *b*, with 24 h post-label incubation in complete medium—(●) detached floating cells; (○) attached monolayer cells—*c*, detached floating cells with 24 h post-label incubation—(●) 8 h incubation with phosphate-buffered medium (PBS) followed by 16 h in complete medium or (○) complete medium (CM) only—*d*, detached floating cells with 48 h post-label incubation—(●) 8 h incubation with PBS or (○) CM only. *e-h*, CHL cells incubated with 0.25 μCi ml⁻¹ for 16 h; *e*, control CHL cells, *f*, treated with distilled water for 5 min and lysed immediately (●) or after 60 min in CM at 37°C (○); *g*, treated with 0.5% deoxycholate for 5 min and lysed immediately (●) or after 55 min in CM at 37°C (○); *h*, treated with a 1:500 dilution of antiserum¹⁰ (○) or with complete medium only (●).

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Alpine Fault of New Zealand and Gondwanaland reconstruction

GRIFFITHS^{1,2} has proposed two reassemblies of Gondwana fragments in the south-west Pacific, including New Zealand and the surrounding complex of submarine ridges and plateaux. Both reconstructions are based essentially on bathymetric data, the salient difference in the later, revised version being a 250 km eastward displacement of Antarctica relative to Australia, to create space for the newly incorporated South Tasman Rise and East Tasman Plateau.

In general terms, Griffiths's reconstructions are feasible, and, as they agree in many respects with my own earlier schematic reconstruction³ of the region, I do not wish to criticise them except on one important issue. This concerns the nature of the Alpine Fault trace in the reconstituted Gondwana landmass. In his original reconstruction, Griffiths¹ achieves a close fit of south-west Pacific continental and quasi-continental crustal segments by introducing a sharp deflection—through some 70°—in the line supposedly followed by the Alpine Fault. In his revised version², the Alpine Fault trace becomes even more complex and describes a neat parabolic curve with bifurcating and reconverging branches. Neither interpretation is consistent with the known geometry and tectonic behaviour of the Alpine Fault, and, in my opinion, both embody misconceptions

arising from a failure to appreciate that dislocation along the Alpine Fault has been accompanied by substantial deformation of the New Zealand crustal segment.

As I have attempted to demonstrate elsewhere³, the distinctive NE-SW trend of the New Zealand landmass and its congruent regional geological strike both relate to New Zealand's evolution, since late Cretaceous times, along the interface between two moving crustal plates. West of New Zealand, the opening of the Tasman Sea has been attributed⁴ to late Cretaceous and Palaeocene activity of a NW-SE trans-Tasman spreading axis (perhaps an initial westward prolongation of the still active Pacific-Antarctic Ridge), that brought about a rapid north-eastward migration of the continental and quasi-continental crust of Lord Howe Rise and Norfolk Ridge. To the south and east of New Zealand, extension of the south-western Pacific Basin commenced simultaneously⁵, and Campbell Plateau and Chatham Rise—their free movement to the north-west obstructed by Lord Howe Rise and Norfolk Ridge—were forced northward at a somewhat slower rate.

These differential movements produced, among other effects, large scale sigmoidal flexuring of the broad belt of Palaeozoic and Mesozoic greywacke formations that had accumulated along the margin of Gondwanaland, in the so-called New Zealand Geosyncline⁶. The development of a complex system of NE-SW major shear fractures, the best known of which is the Alpine Fault, marked the

culmination of this flexuring. Within the vicinity of New Zealand, the original course of the peripheral geosyncline can legitimately be supposed to have been more or less linear, and it is essential that, in any reassembly of crustal fragments, compensation be made for the crustal distortion that followed the disruption of Gondwanaland. Figure 1 demonstrates that, in such reconstructions, it is not sufficient merely to postulate reverse displacement of crustal segments by the amount of obvious fault offset. Admittedly, the structural unravelling of intensely deformed areas is not easy, but I would suggest that allowance for crustal distortion, as well as dislocation, in the New Zealand region would simplify and rationalise the form of the Alpine Fault trace in reconstructions such as those of Griffiths.

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¹ Griffiths, J. R., *Nature*, **234**, 203 (1971).

² Griffiths, J. R., *Nature*, **249**, 336 (1974).

³ Cullen, D. J., *NZ J. Geol. Geophys.*, **13**, 7 (1970).

⁴ Hayes, D. E., and Ringis, J., *Nature*, **243**, 454 (1973).

⁵ Heirtzler, J. R., Dickson, G. O., Herron, E. M., Pitman, W. C., and Le Pichon, X., *J. geophys. Res.*, **73**, 2119 (1968).

⁶ Fleming, C. A., *Tuatara*, **10**, 53 (1962).

DR GRIFFITHS REPLIES—Cullen's main point seems to be that the Alpine Fault is not the only deformation of New Zealand which occurred during seafloor spreading and that it is therefore insufficient simply to reverse the observed 480 km offset in reconstructions. I have, however, always accepted this point; it is not explicitly stated but is quite obvious from the diagrams in my 1971 article (*Nature*, **234**, 203–207). I have suggested (*Nature*, **235**, 83–86; 1972) that the total offset of the New Zealand Geosyncline is some 1,200 km, which is partly taken up by the Alpine Fault, and the rest by 'bending and stretching of the geosynclinal axis'. Though detailed structure is not shown, the general concept is quite clear from the figures. I have also referred to the 'Alpine Fault Zone' as distinct from the specific fault (*Nature*, **249**, 336; 1974); it is the former which is indicated on the figure. I have in preparation an enlarged version of my Alpine Fault model, but as it involves over 30 dia-

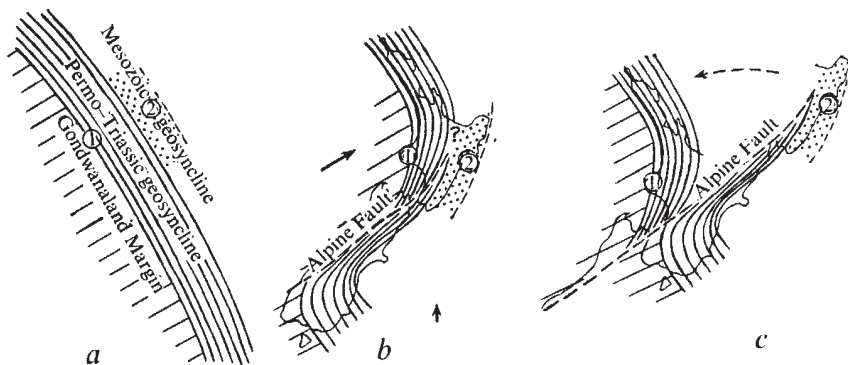


Fig. 1 Schematic reconstruction of the New Zealand region. *a*, Postulated distribution of linear geosynclinal belts along the eastern margin of Gondwanaland. Numerals represent approximate sites of Egmont (1) and Hawke Bay (2). *b*, Present day New Zealand structure. Trend and length of arrows indicate direction and rate of Cainozoic crustal movements. *c*, Spurious Gondwanaland reconstruction (compare Griffiths^{1,2}) achieved by simple reversal of the Alpine Fault displacement and realignment of the Permo-Triassic geosynclinal belt. Note that artificial rotation of the Alpine Fault (broken arrow) places Hawke Bay some 500 km north of Egmont, and duplicates a section of the Permo-Triassic geosyncline.

grams, it will be offered for publication elsewhere. In this model, many of the faults in Southland (for example, the Livingstone and Hollyford faults) are related directly to the distortion which Cullen rightly points out has occurred.

In short, I agree with Cullen's observations that further evidence for crustal distortion should be sought, and that the Alpine Fault with its 480 km offset is only part of the whole story. Apart from this, however, his paper seems to offer nothing positive, and indeed fails to recognise that I have always accepted that such distortion must have occurred. If he has a different regional reconstruction, or a detailed model for the deformation of New Zealand, it might help if this was illustrated by accurate diagrams rather than sketches.

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Dendrochronology and ^{13}C content in atmospheric CO_2

FARMER and Baxter¹ have published $\delta^{13}\text{C}$ values from the wood of tree rings, indicating a decrease over the last 70 yr, which they attribute to the CO_2 increase of the twentieth century. The result, in general, was expected but some implications seem to be questionable, as we can conclude from our results²⁻⁴.

When studying the decrease of ^{13}C in atmospheric CO_2 , wood samples have to be selected very carefully. Samples selected from industrial or forest areas do not give reliable information about the average atmospheric composition⁵. The ideal recorder would be a free-standing tree on a small island far out in the ocean. We analysed tree rings of 10 trees (Fig. 1) and recognised large individual variations both in the absolute data and in the trend.

The processing of the samples is also important. It is well known that a network of resin channels permeates the wood tissue and contains soluble organic matter which cannot be correlated to the age of the wood structure. Furthermore, the components which form the wood, cellulose and lignin, have different isotopic compositions. We found a difference between the $\delta^{13}\text{C}$ values of cellulose and lignin prepared from the same wood, of almost 3‰. The ratio of lignin to cellulose cannot be expected to remain

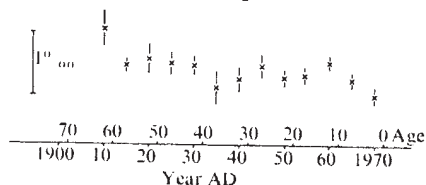


Fig. 1 Average relative ^{13}C decrease in the wood (cellulose) of tree rings during the past 70 yr obtained from 10 free-standing trees; *Quercus robur* (4), *Q. petraea* (1), *Pinus maritima* (2), *Q. lusitanica* (2), *Platanus acerifolia* (1). Each point represents a 5-yr average.

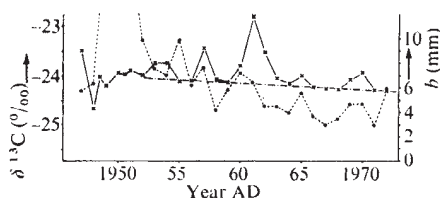


Fig. 2 *Platanus acerifolia* (Azores), showing correlation between ring width, b (dotted line) and $\delta^{13}\text{C}$ (PDB) values (solid line).

constant over the lifetime of the tree⁶, so that the preparation of one pure component is necessary. The chemical preparation of cellulose⁷, if done carefully, does not introduce artificial isotope fractionation.

Another complication arises from the fact that there are normally considerable variations in the ^{13}C content even within one single tree ring of the order of 1‰. That demonstrates clearly the necessity for matching wood taken from the same year but from different positions on the tree. Furthermore, a correlation between ring width (climate ?) and ^{13}C content has been observed (Fig. 2).

Finally, concerning the interpretation of ^{13}C curves with respect to excess CO_2 in the atmosphere, it has to be taken into account that the exchange rate of isotopic CO_2 molecules between the atmosphere and the oceans is faster than the net flux of CO_2 into the oceans (H.D.F. and L.W., and K. Wägener and H.D.F., unpublished). This means that without any further information it is not possible to draw any direct conclusions from ^{13}C measurements about the actual CO_2 excess in the atmosphere, even if the fractionation factors are constant.

A detailed presentation of our results will be given elsewhere.

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- Farmer, J. G., and Baxter, M. S., *Nature*, **247**, 273 (1974).
- Wiesberg, L., and Freyer, H. D., *Tag. Ber. Deut. Miner. Ges.* (February 22, 1973).
- Freyer, H. D., and Wiesberg, L., *Naturwissenschaften*, **60**, 517 (1973).
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- Nikitin, N. J., *The Chemistry of Cellulose and Wood* (Israel Program for Scientific Translations, Jerusalem, 1966).
- Kurschner, K., and Popik, M. G., *Holzforschung*, **16**, 1 (1962).

DRS FARMER AND BAXTER* REPLY—The data of Freyer and Wiesberg¹ represent a welcome and significant contribution to the preliminary study of $\delta^{13}\text{C}$ trends in recent wood. It is gratifying that their results agree closely with our own²

(Fig. 1) and thereby seem reasonably to negate their criticisms of the different procedures. Although suggestive of a common temporal trend in $\delta^{13}\text{C}$, their presentation of data as average deviations over 5-yr periods for 10 trees of differing species and locations does not permit inspection of the individual $\delta^{13}\text{C}$ trend for each tree. They thus preclude any precise assessment of discrepancies caused by variations in species or location. Such discrepancies were, for example, noted in our oak and larch data but, of course, are now obscured by the averaging process.

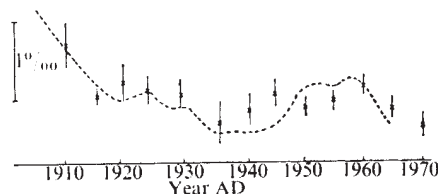


Fig. 1 Comparison of $\delta^{13}\text{C}$ trends in twentieth century wood. Dotted line, mean of data from the same UK oak and larch samples as we reported previously². The points and accompanying errors are those of Freyer and Wiesberg¹ representing the averaged data on 10 selected trees.

In response to specific comments about procedure we would assert that, first, the European larch was indeed selected from a free-standing and exposed situation and yet showed a similar $\delta^{13}\text{C}$ trend to the forest oak. Second, the dangers of inducing fractionation or contamination during cellulose extraction seem comparable to the possible risks in using whole wood. Third, concerning the ever present difficulty in obtaining a representative biospheric sample, the suggestion of Freyer and Wiesberg, though admirable in intention, could well result in a logistical problem of overwhelming proportions without guaranteeing the comparable representativeness of every sample.

Extreme caution in data interpretation was initially urged by us, and Freyer and Wiesberg are correct to reinforce this view. Nevertheless, it remains undeniable that during the first 20–30 yr of this century, recorded levels of $\delta^{13}\text{C}$ show an unexpected decline which could not have been caused even by complete retention of all the fossil CO_2 introduced into the atmosphere. Furthermore, the CO_2 levels deduced from our $\delta^{13}\text{C}$ values for 1900–1920 agree well with those of Callendar's exhaustive summary³, leading to the postulate of an additional biogenic source of CO_2 .

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- Freyer, H. D., and Wiesberg, L., *Nature*, **252**, 757 (1974).
- Farmer, J. G., and Baxter, M. S., *Nature*, **247**, 273–5 (1974).
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Mononuclear cells killing *Cryptococcus*

DIAMOND in his report¹ has presented by no means a new discovery; Aronson and Kletter² have already demonstrated the *in vitro* killing of extracellular *Cryptococcus neoformans* by rabbit monocytes even in presence of normal rabbit serum. Monocytes do not necessarily require the presence of specific antibody to kill *cryptococcus* extracellularly but rather the presence of suitable opsonins which presumably enable the pseudopodia of the phagocytes to adhere firmly to the capsule of this fungus. Rabbit, guinea pig and human sera (but not mouse sera) seem to possess opsonins which are able to induce the phagocytosis of the cryptococci as well as the development of monocyte rings around them which, in turn, can destroy the enclosed parasites.

I feel that Diamond errs when he states that activated macrophages "... provided a preferentially favourable medium for intracellular growth of the fungus" and infers that host macrophages may be involved in the dissemination of the disease³. He drew these conclusions from experiments with human monocyte-derived macrophages activated in *in vitro* conditions with streptokinase-streptodornase and kept in culture for 3-7 d before inoculation of cryptococci. I have observed the extracellular and intracellular killing of cryptococci by murine macrophages (activated either nonspecifically or specifically) in certain conditions: that is, by *in vivo*-activated adherent cells kept in *in vitro* culture for only 2 h before the inoculation of cryptococci in the presence of normal rabbit serum^{4,5}; these sera lacked agglutinating capacity. Prolonged *in vitro* incubation of activated macrophages resulted in a corresponding decrease of their intracellular killing activity.

If phagocyte spreading of the infection in the intact host was involved, as suggested by Diamond, then the administration of drugs like cortisone which are known to promptly depress phagocyte numbers in general should localise this infection. On the contrary, however, it is known that cortisone treatment results in the dissemination of this disease^{6,7}. Diamond's interpretation, that the extracellular killing of cryptococci in his system where $10 \mu\text{g ml}^{-1}$ of cytochalasin B was included to prevent phagocytosis of organisms, was due to antibody-dependent killing by monocytes, may not be exactly correct.

Egg-white lysozyme and various cationic proteins from rabbit polymorphonucleocytes are fungicidal for *C. neoformans*⁸ and the addition of

2-10 $\mu\text{g ml}^{-1}$ of cytochalasin B facilitates the release of lysosomal enzymes from macrophages by exocytosis⁹. Thus the extracellular killing of cryptococci by enzymes released from monocytes simply as a result of their incubation with cytochalasin B is a possibility which cannot be dismissed by the data Diamond presented.

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¹ Diamond, R. D., *Nature*, **247**, 148-149 (1974).

² Aronson, M., and Kletter, J., in *Dynamic Aspects of Host-Parasite Relationships* (edit. by Zuckerman A., and Weiss, D. W.), **1**, 132 (Academic Press, New York and London, 1973).

³ Diamond, R. G., and Bennett, J. E., *Infect. Immun.*, **7**, 231 (1973).

⁴ Sethi, K. K., et al., in *Abstr. Ann. Meet. Am. Soc. Microbiol.*, **131** (1973).

⁵ Sethi, K. K., and Pelster, B., in *Proc. Int. Workshop Macrophage Activation*, Reisenberg, West Germany (Excerpta Medica, in the press).

⁶ Sethi, K. K., Salfelder, K., and Schwarz, J., *Mycopathologica et Mycologia Applicata*, **27**, 357 (1965).

⁷ Salfelder, K., in *Handbuch der Speziellen Pathologischen Anatomie und Histologie* (edit. by E. Uehlinger), **3**, 383 (Springer-Verlag, Berlin, 1970).

⁸ Gadebusch, H. H., and Johnson, A. G., *J. infect. Dis.*, **116**, 551 (1966).

⁹ Davies, P., Allison, A. C., and Haswell, A. D., *Biochem. J.*, **134**, 33 (1973).

DR DIAMOND REPLIES—Sethi seems to have overlooked or misinterpreted the major differences between my studies and those of Aronson and Kletter¹. In contrast to their studies, the fungicidal mechanism which I described requires effector cells which are not necessarily monocytes, as well as specific antibody, but no heat-labile opsonins. The heat-labile opsonins which Aronson and Kletter referred to are almost certainly complement components, as detailed in studies of the role of the classical and alternate complement pathways in opsonisation of cryptococci and in the pathogenesis of cryptococcosis²⁻⁴.

In my study of the possible role of fungicidal activity by non-phagocytic cells, mononuclear cell preparations had 20% or more monocytes mixed with lymphocytes. The effect of heat-labile opsonins was eliminated by heat and the role of other complement components was minimised by the large dilution (1:2,000 or 1:20,000) of antibody. No other serum was present. Unlike the studies of Aronson and Kletter, fungicidal activity occurred only in the presence of antibody, even when effector cells were obtained from four donors who had positive delayed skin tests to cryptococcal antigen.

Sethi's assumption that monocytes are responsible for the fungicidal activity is not warranted. In fact, I

suggested an analogy to antibody-dependent cell-mediated lysis of mammalian target cells, which is known to occur when all phagocytic cells have been eliminated from effector cell populations⁵. In subsequent studies, we used lymphoid cells depleted of phagocytic cells by incubation with carbonyl iron particles and passage through a magnetic field modified from the technique of Lundgren *et al.*⁶. Only 43.5% of a cryptococcal inoculum survived after incubation for 4 h. This fungicidal activity occurred only in the presence of antibody (R.D.D. and A. C. Allison, unpublished).

The role of the macrophage in immunity to cryptococcosis is another issue entirely, and one which is too complex to be thoroughly discussed here. In any case, corticosteroids have a wide range of effects, and I am surprised that Sethi assumes that depression of the number of phagocytes is the overriding corticosteroid effect on the immune response.

I did not study the mechanism of antibody-dependent killing and certainly did not assume that fungi were killed by monocytes, as Sethi states. Monocytes may well have this killing capacity, and lysosomal enzyme release may well be important. Preliminary studies with my coworkers (R.D.C., C. Cardella, P. Davies, and A. C. Allison, unpublished) have shown, however, that supernatants rich in lysosomal enzymes released from macrophages⁷ do not kill cryptococci and that cryptococci induce detectable specific lysosomal enzyme release only in the presence of heat-labile opsonins. Anti-cryptococcal antibody augments this release only slightly, if at all.

I regret that Sethi has misunderstood my work, but appreciate the opportunity to rectify the inadvertent omission of reference to the work of Aronson and Kletter, which was called to my attention after my manuscript appeared.

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¹ Aronson, M., and Kletter, J., in *Dynamic Aspects of Host-Parasite Relationships* (edit. by Zuckerman, A., and Weiss, D. W.), **1**, 132 (Academic Press, New York and London, 1973).

² Diamond, R. D., May, J. E., Kane, M. A., Frank, M. M., and Bennett, J. E., *Clin. Res.*, **21**, 597 (1973).

³ Diamond, R. D., May, J. E., Kane, M. A., Frank, M. M., and Bennett, J. E., *J. Immun.*, **112**, 2260 (1974).

⁴ Diamond, R. D., May, J. E., Kane, M. A., Frank, M. M., and Bennett, J. E., *Proc. Soc. exp. Biol. Med.*, **144**, 312 (1973).

⁵ Holm, G., and Perlmann, P. J., *J. exp. Med.*, **125**, 721 (1967).

⁶ Lundgren, G., Zukoski, C. H. F., and Möller, G., *Clin. exp. Immun.*, **3**, 817 (1968).

⁷ Davies, P., Allison, A. C., Ackerman, J., Butterfield, A., and Williams, S., *Nature*, **251**, 423 (1974).

reviews

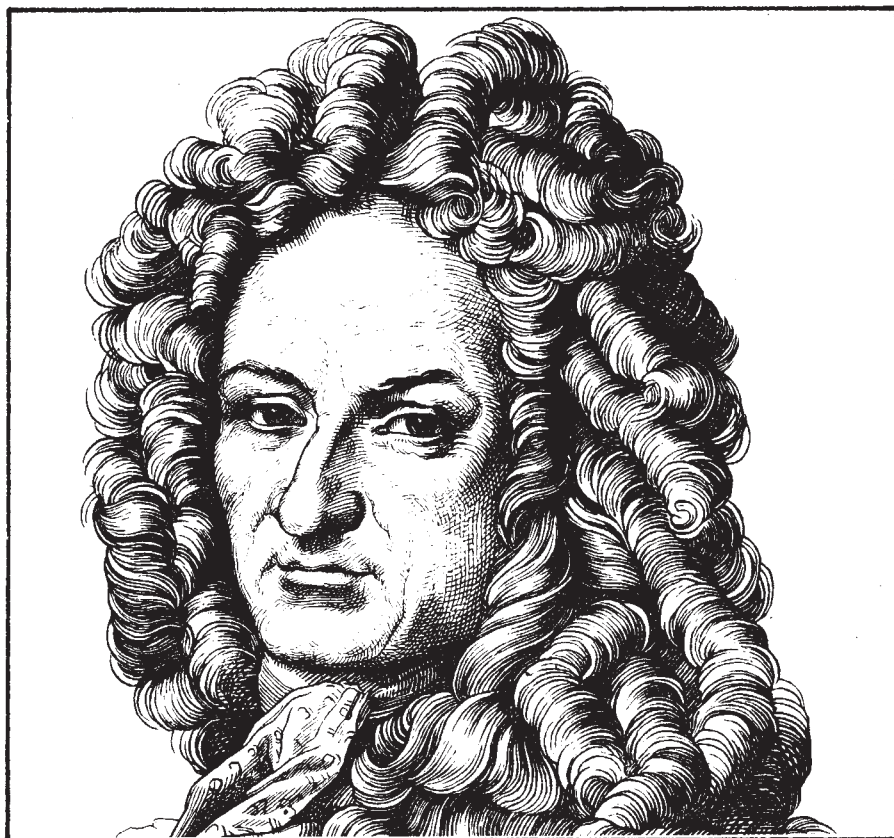
Triangle of parallel ideas

Leibniz in Paris 1672–1676: His Growth to Mathematical Maturity. By Joseph E. Hofmann. Pp. xi+372. (Cambridge University: London, June 1974.) £8.50.

WHEN Leibniz arrived in Paris in March 1672 as the travelling companion of Melchior Friedrich von Schönborn he knew little of the mathematics of the time. But under the guidance of Huygens he made rapid progress and over the next four years conceived his decisive ideas in mathematics, bringing them to a degree of completeness that allows us to see all his later research as elaboration and development. Leibniz would have liked to remain permanently in Paris but having failed to obtain a post there he reluctantly moved to Hanover in October 1676, where he took charge of the Library. It is a testimony to his irrepressible optimism that despite the uncertainty regarding his future Leibniz succeeded, during his last year in Paris, in attaining his greatest mathematical achievement: the invention of the calculus.

Professor Hofmann's brilliant account of these fruitful years in Paris, originally published as *Die Entwicklungsgeschichte Leibnizschen Mathematik während des Aufenthalts in Paris (1672–1676)*, is now presented in a new version, carefully revised by the author and competently translated by A. Prag and D. T. Whiteside. The story is told with the thoroughness, meticulous documentation and clarity of exposition we have come to expect from the lamented master in the field of Leibnizian mathematics. There are three indexes. The first is a chronological index giving a register of all letters or original publications in contemporary periodicals mentioned in the text or footnotes, locations of manuscripts, meetings of learned societies and a checklist of publications in the periodical literature of the time. This is followed by an index of names and works, and a good subject index.

Leibniz's first mathematical discovery in Paris, concerning the summation of infinite series, illustrates two important characteristics of his work, namely the role played by considerations of logic and his preference for methods rather



Gottfried Wilhelm von Leibniz—did not borrow from Newton

than results. Beginning simply with definitions and the axiom of identity, Leibniz obtained from Grégoire de Saint-Vincent's geometrical progression a general method which enabled him to sum the series of reciprocal triangular numbers, a problem proposed to him by Huygens.

Soon after his arrival in Paris, Leibniz made his first visit to London. Although this was not an unqualified success, it marked the beginning of the exchanges with English mathematicians which led eventually to the priority dispute concerning the invention of the calculus. Having appropriated Sluse's tangent rule, Newton maintained that it was the communication of this rule in his own letter that led Leibniz to develop the ideas of the differential calculus. Leibniz always insisted that he had the rule from Sluse, and an excerpt from Sluse's treatise among his papers confirms the truth of this claim. Hofmann effectively demolishes the idea—unquestioned since the suggestion of Tschirnhaus in 1678—of a link between the ideas of Leibniz and those of Barrow. It was not from

Barrow and Newton but from Huygens, Grégoire de Saint-Vincent, Mercator, Gregory and Sluse that Leibniz received his inspiration. When Leibniz met Tschirnhaus towards the end of November 1675, he already possessed his notation for the infinitesimal calculus. It is clear that Tschirnhaus, who had just visited London, could not have transmitted accurate reports of English mathematical methods to Leibniz (even if he had been told anything in detail) for, as his subsequent correspondence shows, he had not really penetrated to any fundamental level of mathematical understanding.

On the documentary evidence so skilfully presented and analysed in this volume, it is clear that Leibniz had truth on his side when he stressed that, as far as methods were concerned, he got nothing from the English. Hofmann's conclusion seems eminently fair: each of the three rivals (Newton, Leibniz and Gregory) achieved his own method; none borrowed or took over, from either of the others, more than certain incidental details.

E. J. Aiton

Phloem transport

Transport of Nutrients in Plants. By A. J. Peel. Pp. 258. (Butterworth: London, April 1974.) £4.80.

THE author's name is a truer guide to the content of the book than the title: transport in the phloem is the subject studied in depth. Discussion of transport in the xylem occupies about a tenth of the book. The symplast system is described, perhaps as an afterthought, in one page of the introduction. Transport across membranes or within the cells is mentioned only briefly. Nevertheless, as a book on phloem transport, this is a most useful work that successfully communicates the nature of scientific investigation and explores a system which is of biological interest to both the academic student and the applied scientist.

Although a self contained book in its own right, the author facilitates further reading by signposting the more relevant recent reviews to augment the information he presents, and by citing some original papers. A glance through the contents rapidly conveys the wide range of background provided. I anticipate that the concepts and approaches discussed will not become rapidly outdated. The book would serve well as an introduction enabling the student to proceed with greater in-

sight to the proceedings, shortly to be published, of the Phloem Transport Conference just concluded at Banff, Alberta.

The text contains remarkably few factual errors, especially for a first edition. It also presents a fair assessment of the current balance of opinion and of the problems associated with interpreting experimental data in relation to theoretical (or hypothetical) models. The evidence quoted by Peel shows a distinct personal bias, but this prerogative may be allowed as it is relevant work which advances the line of the arguments. Data are quoted with a high degree of reliability throughout the text. In the case where the results presented did not seem to fit—a table on page 30—I found that the error arose only because the data were condensed from two separate experiments in the original reference.

There is a short section which lacks the high standard of clarity shown in the rest of the book, in which Peel calculates how much energy, compared with that available from metabolism, would be required to drive actively a volume flow through sieve tubes containing fibrils. He implies that such a flow would be driven against some overall pressure difference, whereas a metabolically dependent pumping mechanism might well drive a flow against a viscous resistance in the absence of an overall pressure difference. His calculation is, of course, correct but the purpose and the understanding are obscured.

These two criticisms are, however, on very rare exceptions in the text. For the advanced undergraduate, as an introduction to further study, and as a background to applied biology, the book conveys a clear and realistic appraisal of the variety of approaches and of the disagreements and controversies within the field of phloem transport.

David Aikman

margins of the Indian plate from Turkey to the Macquarie Ridge examining 20 sections on the way. And if you fancy the Pacific, there are 20 segments of circum-Pacific structures laid out for your consideration.

About 100 scientists have contributed to the making of this book. As a beginning, a small group worked out a questionnaire which could be applied to each selected segment. It posed over 300 questions relating to such matters as surface shape, deep structure, and time relationships, and was designed to elicit a series of factual answers. The main mass of the book consists of accounts of 46 segments based on the responses of over 80 experts who replied to the questionnaire. Dr Spencer collated and edited these replies. On his shoulders and on Sir Peter Kent, who chaired the group which initiated the project, and who in the later stages provided the resources which brought it to completion, has lain the main burden of producing this volume. Readers can judge for themselves how successful the work has been. Coming to the completed book as a member of the group who started the project I think the result is impressive. Much effort separates the finished work from the initial proposals of the planning committee and I do not feel that membership of that body prevents me from congratulating Dr Spencer and Sir Peter. By their efforts they have transformed the original idea—Mr Brian Harland's, as I recollect—into a book of 800 solidly packed pages.

Geotectonic theorists can now contemplate systematic accounts of segment after segment of the Mesozoic and Tertiary fold belts of the world in comfort by the fireside. More to the point, the book provides a useful tool for further research. It supplies what was planned at the outset—data for orogenic studies. It shows that the complexities of mountain chains can be treated in a systematic fashion. The amount of data presented varies from region to region, as is to be expected. More serious is the lack of information from the Soviet Union and the western states of America despite vigorous efforts to fill that gap. The Geodynamics Programme includes, however, a similar but more extensive project which may be expected to compensate for those omissions.

Of particular interest is the opportunity this volume provides for comparisons between the make up of young fold belts and structures formed earlier in the history of the Earth. There is a growing body of evidence which suggests that global tectonics have changed with time. Data such as this should help to show whether or not that view is firmly founded. J. Sutton

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Younger fold belts

Mesozoic-Cenozoic Orogenic Belts. Data for Orogenic Studies. Edited and collated by A. M. Spencer. Pp. xvi+809. (Scottish Academic Press: Edinburgh, 1974.) Published for The Geological Society, London. n.p.

THIS book tells the reader about some 40 segments selected from fold belts formed since Mesozoic times. Dr Spencer writes in his introduction that the volume is not designed for fireside reading, but I rather doubt that. It sets out to provide an objective presentation of what is known about each region; as far as possible the treatment is uniform from sector to sector. The result I find is absorbing and the book rather well suited for the evening fireside. With it one can travel along the

Animals' sticking places . . .

Biological Mechanisms of Attachment. By W. Nachtigall. Translated by M. A. Biederman-Thorson. Pp. viii+194+53 plates. (Springer-Verlag: Berlin and New York, 1974.) DM75; \$30.60.

THIS book is an oddity. As description in hard engineering terms of the ways in which creatures of all kinds attach themselves to their prey, substrate, or each other, it can have little practical value. So it must be read, presumably, for the enjoyment of seeing how nature's engineering parallels that of man.

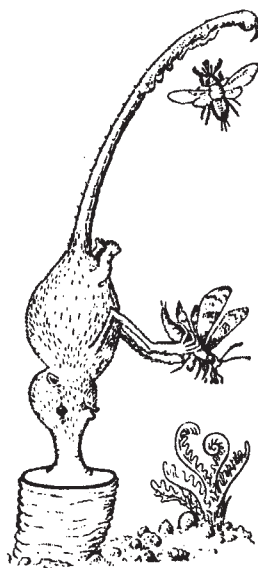
The book is not an exhaustive catalogue (Dr Nachtigall does not describe the flexible limb joints of animals, for example) but it includes devices which hook, grip, clamp, glue, suck or interlock organs or individuals together in permanent or temporary bonds. Any book of this kind is necessarily a list to be dipped into rather than a text to be read from cover to cover, so it is just as well that most of the 712 illustrations are arranged on pages which are opposite their explanations in the text. Where possible, the solutions that human engineers have come up with to meet the same circumstances are described and it is curious how alike many of them are.

Dragonflies, for example, which mate on the wing face problems similar to those of aircraft refuelling in flight, and the mating of the *Aedes* mosquito (where a perfect alignment of the reproductive tracts must be made and broken in about 14–20 seconds) resembles the 'hard docking' of spacecraft.

Suction seems to be the most widespread attachment mechanism throughout the phyla and about one third of Dr Nachtigall's book is devoted to it. Examples range from the sucking mouthparts of helminth parasites to the suction pads on the feet of the tarsier.

Although arthropods and helminths tend to dominate the book, many old favourites like the pop fastener on the mantle of the squid also appear. The head of the tapeworm, the claw of the arthropod and the Aristotle's lantern of the seurchin are also rather obvious examples. This indicates a rather subtle drawback of the book: most of the contents, although admirably explained and compared, could probably be guessed by any competent biologist.

There are some surprises—for example, the revelation that no two tissues are ever connected in nature by a third, like a nail or screw—but the merit of the book must remain its value



The fanciful monorhina, Dulcicauda grisegrelli

for directing the biologist to think of the attachment mechanisms of animals in engineering terms rather than as any sort of reference work.

The author makes two curious mistakes. The first (and most serious) is that the text describing the attachment of the sucker organ of the remora does not relate to the series of drawings used to illustrate it—indeed it describes the opposite (and wrong) sequence of events. The second is that the author has placed a charming (but unreal) member of the Monorhina in a section dealing with suction cups when, of course, it uses an adhesive.

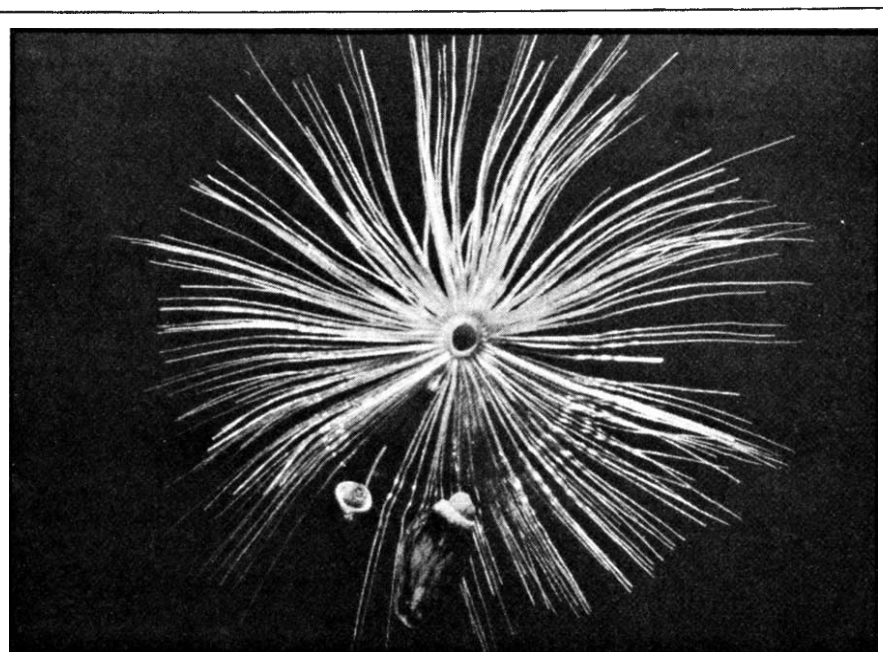
John Wilson

. . . placing sticking plants

Mycologists Handbook: An Introduction to the Principles of Taxonomy and Nomenclature in the Fungi and Lichens. By D. L. Hawkesworth. Pp. 231. (Commonwealth Mycological Institute: Kew, 1974.) £5.50; \$14.30.

MOST biologists have only a superficial knowledge and understanding of taxonomic procedures, and those with a real feeling for the subject are few in number. This book goes a long way towards bridging the gap between the many and the few. The author is to be congratulated on producing a detailed, accurate and thoroughly readable book on a subject which is often dismissed as dull and uninteresting.

Although the book is essentially a guide to budding taxonomists it has much to say to others who experiment with, and publish data on, fungi and lichens. The need to identify material correctly, often a difficult task with fungi and lichens, and the need to store specimens in accessible herbaria are particularly emphasised; in the past the frequent failure to do this properly has reduced the value of much published work. Topics covered include the collection and preservation of material; taxonomic ranks; the naming and describing of new taxa; nomenclature; information on authorities and herbaria; and information on the abbreviations of the titles of publications not quoted in the World List of Scientific Publications. B. W. Ferry



A somewhat decorative method of dispersal. The fruit and pappus of the thistle *Silybum marianum*. From *The Natural History of New Zealand: An Ecological Survey*. Edited by Gordon R. Williams. Pp. xviii + 434 + 40 plates. (Reed: Wellington, Sydney and London, 1973.) £12.95.

Answers to Monod

Beyond Chance and Necessity. Edited by John Lewis. Pp. xi+141. (Garnstone: London, 1974.) £2.95.

WHEN Jacques Monod, in *Chance and Necessity*, classed Christians and Marxists, along with witch doctors, as "animists" who attribute a will to nature, a reply was to be expected. These essays, published as part of the Teilhard Study Library, are part of that reply; *Beyond Chance and Necessity* brings together a number of people who have little in common other than their disagreement with Monod.

Monod argued that our present cultural confusion arises because our ethical beliefs are based on various pictures of the world, in particular the Christian myth, which ascribes a purpose to the Universe and a special role for man in that purpose, whereas our economic existence depends on the scientific world picture, which does not admit explanations in terms of purposes or final causes, and which sees man as the accidental consequence of natural selection acting on random mutation. He urges us to recognise the distinction between 'objective' statements proper to science, and statements of value which cannot be derived from science. The 'principle of objectivity'—the avoidance of teleological explanation in science—he sees as itself a moral commitment, necessary for the practice of science and, therefore, not derivable from it.

Mary Warnock accuses Monod of arguing in a circle. If science is based on the assumption that there are no purposes in the Universe, one cannot then deduce from science that there is no purpose, and no special role for man. Monod's picture of the world is, in fact, quite consistent with God as a 'first cause', who created a world capable of generating life, and who even retains an interest in that world. This argument is taken further from a Christian standpoint by Arthur Peacocke. He argues, by analogy with statistical mechanics, that the randomness of mutation does not mean that there are no laws of evolution; a point made by other contributors, and one I would accept. For a Christian, however, God as a first cause who designed the laws of nature and the initial conditions to hold the potentiality of life and who then let the Universe run, is not enough. Peacocke is critical of the concept of a "God of the gaps", invoked to explain those phenomena which science cannot, because that can lead only to a dwindling role for God. Instead he sees God as imminent in all natural processes, working through the laws of nature and not by suspending them.

My difficulty here is not so much that I disagree with him as that I cannot see what he can possibly mean. If God is simply another name for the laws of nature, then not only is there no need for that hypothesis, there is no need for the term itself.

The defence of Marx is taken up by John Lewis. It is perfectly possible to be a good philosopher without knowing any biology; unhappily, Dr Lewis writes as if he understood biology when it is quite clear that he does not. That gives such an air of insincerity to his essay that I found it impossible to pay serious attention to it. That is a pity, because I think he is right in arguing that Marx and Engels were not animists in Monod's sense. They saw 'dialectical laws' not as a mind or purpose in nature, but as observable regularities in the behaviour of natural systems.

Joseph Needham also comes to the defence of Marxism. He quotes with approval the antimethodist views expressed by Soviet Marxist scientists in the early 1930s. That will not quite do. A few years later, Marxist philosophers, arguing that the gene was an undialectical entity because it controls development without being influenced by it, supported the destruction of biology in the Soviet Union; at the same time, men who believed that the cell is a machine (not, it is true, a clock—more a tape-recorder) were revolutionising biology.

The most interesting essays in the book are written from within evolution theory and molecular biology, by C. H. Waddington and Robin Monro. Waddington was provoked into contributing by a report that Monod has accused him of Lysenkoism. I do not know whether the report is true, but I am glad of the result; if a man is accused of Lysenkoism it does concentrate the mind wonderfully. Waddington gives an unambiguous account of the significance of genetic assimilation. Monro criticises Monod's excessive reliance on molecular concepts in biology. He first argues that the 'central dogma'—that information cannot pass from protein to nucleic acids—is not necessary for the truth of Weismannism. An 'acquired character' is not usually reflected in a changed amino acid sequence in protein, so even if the central dogma were false, most acquired characters could not be transmitted. Monro goes on to argue that the dogma is not sufficient either, because some kind of germinal selection may produce Lamarckian effects (the same idea is worked out in more detail by Waddington).

The main difficulty in assessing this controversy is that at least two separate issues are being confused. The first is the relationship between science and

ethics. There, I think Monod was right in saying that the scientific world picture has been built by abjuring final causes. I have little sympathy with those of his critics who dislike mechanistic theories in science because they lack moral uplift. But there is second issue, concerning the strategy of scientific research; there, I think Monod's critics have a case. I do not think it will prove possible to explain biology in molecular terms in quite the way Monod hopes. For example, he writes "personally I am convinced that in the end only the shape-recognising and stereospecific binding properties of proteins will provide the key to these phenomena" (that is of development). My own guess is that it will no more be possible to understand the shapes of embryos in terms of the properties of proteins than it is possible to understand the shapes of the waves generated in Jabotinski's reaction in terms of the shapes of the constituent molecules. Of course, theories of development will be dependent on and consistent with molecular biology but they will not be discovered by thinking only at the molecular level.

This guess may be wrong. But then, I wish I was as sure of anything as Monod is of everything. How a man can write "This central concept of modern biology is no longer one among other possible or even conceivable hypothesis, the only one compatible with observed and tested fact. And nothing warrants the supposition (or the hope) that conceptions about this should, or ever could be revised", and claim to be a Popperian, I do not know.

John Maynard Smith

Critical look at the LMFBR

The Liquid Metal Fast Breeder Reactor. By Thomas B. Cochran. Pp. xiv+271. (Johns Hopkins University: Baltimore and London, 1974.) \$6.95.

I CANNOT do better than quote from the preface:

"In this monograph Thomas Cochran takes a critical look at the economic and environmental arguments which have been made in favor of an early introduction of the liquid metal fast breeder reactor (LMFBR) as a central component of the United States electrical energy system."

Taking a number of published United States (US) reports, particularly the cost/benefit analyses put out by the US Atomic Energy Commission in 1970 and 1972, he considers in reasonable detail the choice of discount rate, the assessment of generating costs, the validity of currently assumed perform-

ance parameters, fuel cycle costs, and the arguments relating to the supply and demand of uranium.

Cochran may be right in parts of his criticism, but recent history has shown the need for frequent revision both of the ground rules in policy analyses and of the assumptions that form the background to them. In keeping with his pessimistic view of the LMFBR he suggests the continued use of thermal reactors and the stock-piling of the depleted uranium for future use—may be not with LMFBRs. He advocates further investigation of alternative energy sources, without offering much hope of better success in any other direction.

That part of his submission which challenges the proposal to build many fast reactors between 1986 and 2000 is perhaps less important in terms of today's decision than his critical comparison between the LMFBR and the light-water reactor. Neither in the US nor the UK do we yet know what new information and experience will be available by 1986, and the present need is to ensure a capability of energy production in the latter part of this century. To that end the UK, France, Germany, Italy, Japan and the Soviet Union are actively developing the LMFBR—a system with which Europe has considerably more experience than the United States.

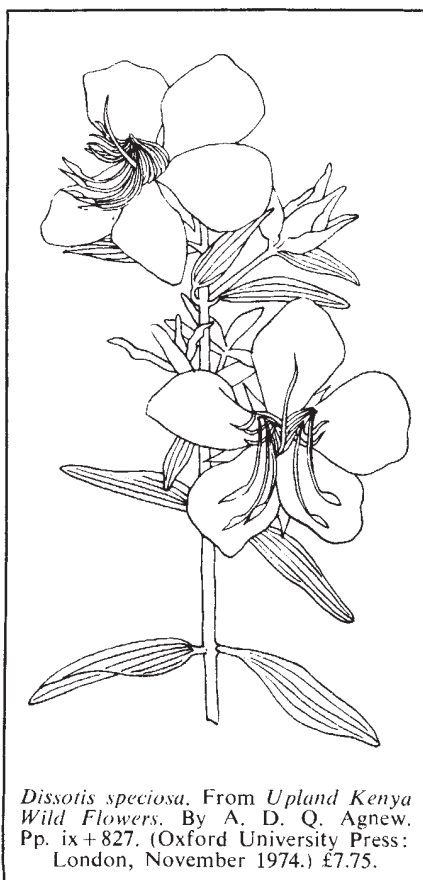
Many of the problems Cochran raises are common to any programme of nuclear power, such as the long-term storage of radioactive waste and the processing and transport of fuel. These become more acute with the rate of increase of the total power programme and are little affected by the choice of systems.

Mr Cochran has made an excellent attempt to analyse the safety of the fast reactor in simple terms. I wish to comment only on a few exceptions: for example, the difference in neutron lifetime is barely relevant to most accident conditions as the rate of change of temperature is dictated by the doppler coefficient, that is, controlled by the thermal inertia of the fuel. A prompt, critical situation cannot be controlled by mechanical systems, neither in thermal nor in fast reactors. The importance of the sodium void coefficient arises only if coolant is ejected from a substantial number of fuel channels. The way in which an accident might develop will be different in different reactor systems but the end effect of the potential thermal interaction of hot fuel and coolant can be violent in water reactors as well as in the LMFBR and the available energy is not markedly different.

There is a growing recognition that there is some uncertainty and consequent risk in many industrial activi-

ties, not only in the development of nuclear power, and it is obviously important to be aware of these risks in order to minimise them. This monograph should encourage further critical reviews of the US programme and the part to be played by fast reactors in a future energy economy.

F. R. Farmer



Dissotis speciosa. From *Upland Kenya Wild Flowers*. By A. D. Q. Agnew. Pp. ix + 827. (Oxford University Press: London, November 1974.) £7.75.

Happy union in the feldspar family

Feldspar Minerals. Vol. 1: *Crystal Structure and Physical Properties*. Pp. xx + 627. Vol. 2: *Chemical and Textural Properties*. Pp. xi + 690. By J. V. Smith. (Springer-Verlag: Berlin and New York, 1974.) Vol. 1: DM98.30; \$40.10. Vol. 2: DM103.50; \$42.30.

THESE splendid volumes on the most abundant, ubiquitous and internally complex minerals in the Earth's crust set a new standard for advanced mineralogical texts. They are extraordinarily comprehensive and the reader must feel a sense of wonder at the author's capacity to organise so vast a body of literature into a coherent and remarkably readable whole. The hundreds of references are very up to date (1973) and much use is made of preprints and theses. The author's aim is avowedly pedagogic, to use the feld-

spars to show how physical and chemical principles can be combined with geological observations to contribute to our understanding of mineral genesis. The books therefore contain several crisp but useful sections outlining most of the many physical and analytical techniques applicable to crystalline materials in general, with many references to basic papers which develop physicochemical principles. At the other extreme, volume 2 covers at length a multitude of petrological observations on feldspar twinning and intergrowths. In their marriage of crystallographic sophistication with grass-roots geological observation (a union sadly missing in much feldspar research) the books set an example applicable across the whole mineralogical field.

The volumes are so comprehensive that feldspar specialists are likely to find their own field dealt with at length, with a most valuable review of all the relevant literature; for more general readers certain sections stand out. In volume 1 the structural architecture of the feldspars is developed in great detail, beginning with discussion of models of bonding. The unsolved intricacies of the one or two-step ordering problem in K-feldspars are discussed and later related to the difficulties of classification of the K-feldspars. These sections as nomenclature are required reading for petrographers, who will also welcome the compilation of X ray and optical determinative techniques. Electron-optical methods will also find increasing use as a petrographer's tool and photographs of many fascinating (and sometimes enigmatic) textures are reproduced clearly.

Volume 2 begins with an outline of analytical techniques followed by a lengthy description of chemical substitution. Mechanisms of growth, development of chemical zoning, morphology and all the many feldspar twins are described, with structural explanations and a widely applicable discussion of twinning mechanisms. Similarly, intergrowths of feldspar with feldspar and with a host of other minerals are explained in terms of mechanisms of exsolution and replacement. Volume 3, dealing with phase equilibria and petrogenesis (promised for 1976) must be awaited eagerly by all mineralogists and petrologists. These lavishly produced, totally comprehensive volumes bring out, through the author's obvious enthusiasm, all the fascination there is in using modern techniques to explain mineral variation and it is to be hoped that they will be treated not just as invaluable works of reference, but also as a guide to all that is possible in modern mineralogical work.

Ian Parsons

announcements

Appointments

Francis Joseph McQuillin has been appointed to a personal chair of organic chemistry at the **University of Newcastle-upon-Tyne**.

George Albert Swan has been appointed to a personal chair of organic chemistry at the **University of Newcastle-upon-Tyne**.

Awards

J. Baddiley has been awarded the **Davy Medal** by the **Royal Society** for his work in the field of chemistry.

W. G. Richards has been awarded the **Butterworths Scientific Fellowship for Authors** for 1975.

International meetings

February 3–7, **1975 National Symposium on Forensic Sciences**, Perth (Mr V. J. McLinden, Secretary, Australian Forensic Society, Government Chemical Laboratories, 30 Plain Street, Perth, Western Australia 6000).

February 4, **Entry and Distribution of Pollutants in Groundwater and Rivers**, London (The Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS).

February 6, **The Role of Toxicology in the Development of a Drug**, London (Dr J. F. Cavalla, John Wyeth & Brother, Ltd., Huntercombe Lane South, Taplow, Maidenhead, Berkshire).

February 17–18, **Glacial Till**, Ottawa (M. K. Ward, Executive Secretary, Conference on Glacial Till, c/o National Research Council of Canada, Ottawa, Canada K1A 0R6).

February 24–27, **4th Conference on Experimental Medicine and Surgery in Primates**, Jerusalem (The Secretariat, P.O.B. 16271, Tel Aviv, Israel).

March 3–7, **26th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy**, Cleveland, Ohio (Mr S. David Cifralak, Calgon Corporation, Pittsburgh Activated Carbon, PO Box 1346, Pittsburgh, Pennsylvania 15230).

March 4, **Aspects of Fungicidal Selectivity**, London (The Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS).

March 10–15, **Dahlem Workshop on the Nature of Seawater**, Berlin (Dahlem Konferenzen, 1 Berlin 33, Delbrückstrasse 4c, Germany).

March 13–14, **Symposium on Infections of the Foetus and Newborn Infant**, New York (Dr Saul Krugman, New York University Medical Center, School of Medicine, 550 First Avenue, New York, N.Y. 10016).

March 13–15, **Energy Transducing Membranes: Structure, Function and Reconstitution**, Buenos Aires (Dr Alberto A. Boveris, Universidad de Buenos Aires, Facultad de Medicina, Instituto de Química Biológica, Paraguay 2155, Buenos Aires, Argentina).

March 17–21, **Biological Microanalysis**, Paris, (Dr Patrick Echlin, Biological Microprobe Laboratory, Department of Zoology, Downing Street, Cambridge CB2 3EJ, UK, or Professor Pierre Galle, Faculté de Médecine de Créteil, 6 rue du Général-Sarrail, 94010 Créteil, France).

March 24–25, **Zmuda Memorial Conference on Geomagnetic Field Models**, Colorado Springs (American Geophysical Union, 1707 L Street, NW, Washington DC 20036).

March 24–26, **Europhysics Conference on Nuclear Interactions at Medium and Low Energies**, Harwell (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

March 26, **The Structure and Origin of Comets**, York (Professor M. M. Woolfson, Department of Physics, University of York, Heslington, York YO1 5DD).

March 31–April 3, **5th International Symposium on Flavins and Flavoproteins**, San Francisco (Dr Thomas P. Singer, Molecular Biology Division, Veterans Administration Hospital, 4150 Clement Street, San Francisco, California 94121).

Reports and publications

Great Britain

Annual Report of the Scottish Marine Biological Association for the year ending March, 31, 1974. Pp. 69. (Oban, Argyll: Scottish Marine Biological Association, 1974.) 75p. [710]

Keyword Index of Guides to the Serial Literature. By A. G. Myatt. Pp. 35. (Boston Spa, Wetherby: The British Library, Lending Division, 1974.) £1. [810]

Science Research Council. Greenwich Time Report. Royal Greenwich Observatory Time and Latitude Service, 1973 July–December. Pp. 369–396. (London: Science Research Council, 1974.) [1010]

Imperial College of Science and Technology. (University of London). Safety Precautions in the Use of Electrical Equipment. Second edition. Pp. 24. Code of Practice Against Radiation Hazards. Sixth edition. Pp. 72. Precautions Against Biological Hazards. Pp. 119. Safety in Chemical Laboratories and in the Use of Chemicals. Third edition. Pp. 46. (London: Imperial College of Science and Technology, 1974.) [1010]

The Hostile Environment of Man (Report of a Symposium held on May 1, 1971, at the University of Leeds.) (The Journal of the Royal College of General Practitioners, Supplement No. 1, Vol. 24, 1974.) Pp. 46. (London: The Royal College of General Practitioners, 1974.) £1.25. [1010]

Proceedings of the Royal Irish Academy. Vol. 74, Section A, No. 13: Power Mappings and Group Morphisms. By D. MacHale. Pp. 91–94. 14p. Vol. 74, Section B, No. 16: The Ecology of Collembola in Irish Blanket Bogs. By R. E. Blackith. Pp. 203–226. 38p. Vol. 74, Section B, No. 17: The Stratigraphy of the Carboniferous Rocks of Hook Head, Co. Wexford. Pp. 227–244. 33p. (Dublin: Royal Irish Academy, 1974.) [1010]

Other countries

United States Department of the Interior: Geological Survey. Professional Paper 829: Geology and Mineral Deposits of the Poncha Springs SE Quadrangle, Chaffee County, Colorado. By Ralph E. Van Alstine. Pp. iii + 19 + plate 1. (Washington, DC: Government Printing Office, 1974.) \$1.95. [309]

Smithsonian Contributions to Botany. No. 14: Commercial Timbers of West Africa. By Edward S. Ayensu and Albert Bentum. Pp. iii + 69. \$1.65. Smithsonian Contributions to Anthropology. No. 18: Reconstruction of Demographic Profiles from Ossuary Skeletal Samples—a Case Study from the Tidewater Potomac. By Douglas H. Ubelaker. Pp. xi + 79. \$2.25. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) [110]

Transactions of the American Philosophical Society. New Series. Vol. 64, Part 4: Mappae Clavicula—a Little Key to the World of Medieval Techniques. By Cyril Stanley Smith and John G. Hawthorne. Pp. 128. (Philadelphia: The American Philosophical Society, 1974.) \$7. [110]

East African Community. East African Virus Research Institute Report for 1972. (No. 22). Pp. ii + 56. (Entebbe, Uganda: East African Virus Research Institute, 1972. Shs. 12. [210]

Theoretische Aspekte der Menschwerdung. Von B. Rensch und J. L. Franzen. (Aufsätze und Reden der Senckenbergischen Naturforschenden Gesellschaft.) Pp. 59. (Frankfurt am Main: Verlag Waldemar Kramer, 1974.) DM. 20. [210]

Lectures in Plasma Physics: The Magnetohydrodynamic Approach to the Problem of Plasma Confinement in Closed Magnetic Configurations. By C. Mercier, in collaboration with H. Luc. (Research Group of the Association EURATOM-CEA, CEN/Fontenay-aux-Roses (France). Pp. (Luxembourg: Commission of the European Communities, 1974.) [310]

Professional Profile. Vol. 1, No. 1, 1974. Pp. 1–36. (Eindhoven, The Netherlands: NV Philips Gloeilampenfabriek, 1974.) [410]

Canada: Department of Energy, Mines and Resources. Economic Geology Report No. 29: Niobium (Columbium) and Tantalum in Canada. By K. R. Dawson. Pp. ix + 157. \$4.50. Paper 74–22: Gravity and Magnetic Natural Resource Maps (1972) Offshore Eastern Canada—Philosophy and Technique in Preparation by Computer. By Richard T. Haworth. Pp. 15. \$3. Paper 74–24: *Calliergon oftianum* Steere in Late Tertiary and Pleistocene Deposits of Canada. By M. Kuc. Pp. 6. (2 plates). \$2. Paper 74–32: Geochemical Studies in the Surficial Environment of the Beaverlodge Area, Saskatchewan. By Willy Dyck. Pp. 30. \$3. Paper 74–45: Elemental Associations of Mineral Deposits and Indicator Elements of Interest in Geochemical Prospecting (Revised). By R. W. Boyle. Pp. 40. \$2. Paper 74–52: Field Data Acquisition Methods for Applied Geochemical Surveys at the Geological Survey of Canada. By Robert G. Garrett. Pp. 36. \$3. (Ottawa: Information Canada, 1974.) [410]

Environment Canada: Fisheries and Marine Service. Technical Report No. 466: An Attempt to Overwinter Salmonids at Prevailing Seawater Temperatures in New Brunswick. By R. L. Saunders, B. C. Muise and E. B. Henderson. Pp. 14. Technical Report No. 480: User's Manual for Pisces—a General Fish Population Simulator and Fisheries Program. By A. V. Tyler. Pp. 30. (St. Andrews, N.B.: Research and Development Directorate, Biological Station, 1974.) [710]

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Minerals Research Laboratories, 1973/1974. Pp. 71. (Melbourne: CSIRO, 1974.) [710]

Practical Building of Methane Power Plants for Rural Energy Independence. By L. John Fry. Edited by D. Anthony Knox. Pp. 96. (Santa Barbara, California: Standard Printing, 1974.) [910]

Journal of Environmental Economics and Management. Vol. 1, No. 1, May 1974. Pp. 1–88. Edited by Allen V. Kneese and Ralph C. d'Arge. Subscription: Vol. 1 (4 issues), 1974, \$30. (New York and London: Academic Press, 1974.) [710]

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Plant Industry, 1973. Pp. 171. (Canberra: CSIRO, 1974.) [710]

United States Department of the Interior: Geological Survey. Professional Paper 830: Zeolites and Associated Authigenic Silicate Minerals in Tuffaceous Rocks of the Bog Sandy Formation, Mohave County, Arizona. By Richard A. Sheppard and Arthur J. Gude. 3rd. Pp. iv + 36. (Washington, DC: Government Printing Office, 1973.) \$1.05. [710]

New Zealand. Report of the Department of Scientific and Industrial Research for the year ended March 1974. (C21). Pp. 68. (Wellington: Government Printer, 1974.) 45c. [810]

United States Department of the Interior: Geological Survey. Professional Paper 772: Gold-Bearing Gravel of the Ancestral Yuba River, Sierra Nevada, California. By Warren E. Yeend. Pp. iv + 44 + 2 plates. (Washington, DC: Government Printing Office, 1974.) \$2.70. [810]